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Effect of temperature and light intensity on early development of African sharptooth catfish in commercial production

Vliv teploty a intenzity světla na raný vývoj sumečka afrického v komerčním chovu

Markéta Prokešová

Czech Republic, Vodňany, 2016

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1.1. AFRICAN SHARPTOOTH CATFISH

The African sharptooth catfish, *Clarias gariepinus* (Fig. 1) belongs to the exceptionally diverse order Siluriformes (catfishes) that is divided into 36 families and 477 genera distributed worldwide. The group of catfishes includes 3088 freshwater and marine fish species from small aquarium fishes (as family *Callichthyidae*, less than 10 cm) to large fish species (up to 330 kg) of economical importance, as *Ictalurus punctatus* (Rafinesque, 1818), *Silurus glanis* (Linnaeus, 1758), *Pangasius hypophthalmus* (Sauvage, 1878), *Silurus asotus* (Linnaeus, 1758), or *Clarias gariepinus* (Lundberg and Friel, 2003; Orban et al., 2008; Nirchio et al., 2010; FAO, 2016).

Figure 1. *African sharptooth catfish – Clarias gariepinus (Photo by M. Prokesova).*

As other catfishes from the family Clariidae (Fig. 2), the African sharptooth catfish is characterized by the presence of the unique arborescent suprabranchial organ formed by the second and the fourth gill arches, which allows it to breathe atmospheric oxygen (Viveen et al., 1986; de Graaf and Janssen, 1996; Appelbaum and Kamler, 2000; Kurka et al., 2000; Teugels, 2003; Teugels and Adriaens, 2003; Agnese and Teugels, 2005). Naturally, the African sharptooth catfish occurs in subtropical and tropical fresh waters – lakes, rivers and swamps in Africa and Southwest Asia (Hecht et al., 1988; Agnese and Teugels, 2005; Kouril et al., 2013; FAO, 2016). It is nocturnal tactile-chemoreceptive predator (Viveen et al., 1986; Hecht and Appelbaum, 1988) which feeds especially at the bottom (Teugels, 1986) on a wide variety of prey (Burgess, 1989), like insects, plankton, invertebrates and fish, but also takes in young birds, rotting flesh and plants (de Moor and Bruton, 1988; Hamackova et al., 2007).

This fish species is an ideal candidate for intensive aquaculture. It can be reared at high stocking densities of 300–400 $kg.m^{-3}$, in water with low oxygen level, high ammonia concentration and organic pollution. It is high resistant to diseases and parasites. Besides, it is characterized by rapid growth, quality meat (high protein and low lipid content) without intramuscular bones. Furthermore, artificial spawning of females can be induced several times a year (Teugels, 1986; Appelbaum and McGeer, 1998; Appelbaum and Kamler, 2000; Adamek et al., 2011; Kouril et al., 2013; FAO, 2016). Nevertheless, they suffer high mortality in early life stages because of low fertilization rate (Hamackova et al., 2007), low hatching and survival rates (Muchlisin et al., 2010), cannibalism among its larvae and juveniles (Hecht and Appelbaum, 1988; Smith and Reay, 1991; Hecht and Pienaar, 1993; Mukai and Lim, 2011), and difficulties of larvae to adjust to a formulated diet (Verreth and Van Tongeren, 1989; Verreth, 1994).

The worldwide aquaculture production of African sharptooth catfish has increased from 5430 tonnes to 237124 tonnes (more than forty-fold) since 2000 to 2014, respectively

(FAO, 2016). Whereas, the global capture fisheries of African sharptooth catfish fluctuated from 39537 tonnes to 49123 tonnes between 2003 and 2012, respectively (FAO, 2014). The largest producers of captured and farmed African sharptooth catfish are African countries (especially Nigeria, Uganda, Mali, Ethiopia and Kenya). The African sharptooth catfish is farmed in some Asian and South American countries (Ponzoni and Nguyen, 2008; FAO, 2014, 2016). Also, it was introduced in the Netherlands in 1977 from where it was transferred to some other European countries (Hungary, Germany, Poland, the Czech Republic and Belgium). Production remains significant only in Hungary (1852 tonnes in 2012) and the Netherlands (1200 tonnes in 2012), according to FAO (2014, 2016) and Peteri et al. (2015).

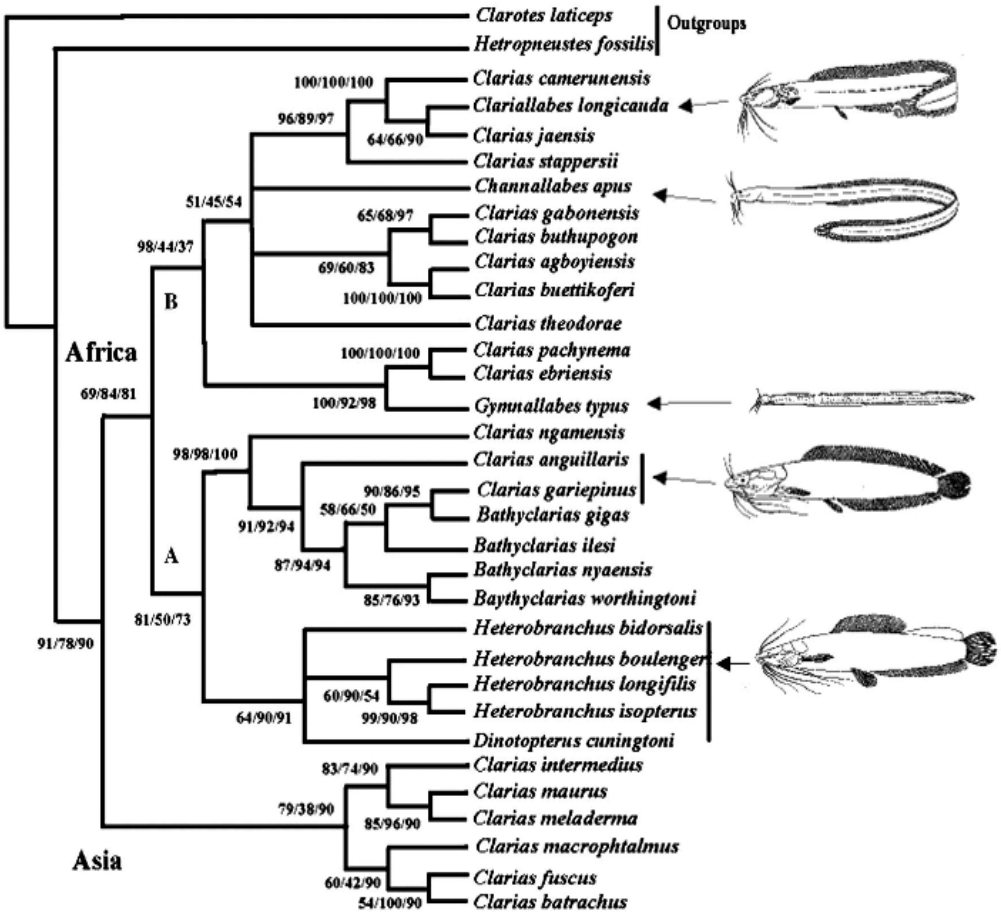

Figure 2. *Geographic origin of Clariidae catfishes is divided into African and Asian species. In African species, two different lineages (A and B) are recognized. Group A represents the "big head and numerous fins" species. Whilst, group B includes "eel-like" species (Agnese and Teugels, 2005).*

The African sharptooth catfish is the most important fish species in Africa besides tilapias and its market demand is continuously increasing. However, in Europe, it is only a supplemental fish species that adds to the variety in the fish market, and its local as well as foreign market demands are extremely fluctuating (Ponzoni and Nguyen, 2008; FAO, 2016). For instance, the import of filleted pangasius catfishes from Southeast Asia had unfavourable

effect on both production and consumption of Arican sharptooth catfish in Europe resulting in a decrease of production between 2009 and 2015 (Peteri et al., 2015). Generally, the grow-out systems for rearing of African sharptooth catfish vary according to the climate, location, scale of production, and level of regional development. This species is usually reared in pond systems or cages in countries of its natural distribution and suitable climate, whereas, at the places of its artificial introduction (including the Czech Republic), it is usually farmed in flow-through and indoor recirculating aquaculture systems (RAS) with a possible summer breeding in ponds, or with using of geothermal water (Fleuren, 2008; Ponzoni and Nguyen, 2008; Peteri et al., 2015; FAO, 2016).

In the coming 50 years, the global aquaculture production is expected to grow about five-times (Avnimelech et al., 2008). There has been a rapid increase in production of salmon, shrimp and catfish due to globalizing trade and favourable economics of larger scale intensive farming (Bostock et al., 2010). The African sharptooth catfish also might be a suitable candidate to contribute to the fulfilment of a similar increase in demand. Especially, there is still a high potential for production of African sharptooth catfish in African countries despite, the fact that the industry has not been well established there. Several factors are limiting, such as a serious lack of good quality seedstock, difficulties with artificial reproduction of male broodstock, low survival rates of early stages, lack of least-cost optimally balanced diet from local sources, poor management practices, and a poorly developed processing and marketing systems (Fleuren, 2008; Ponzoni and Nguyen, 2008).

1.2. REPRODUCTION

In natural conditions, the African sharptooth catfish has a cyclical and seasonal gonadal maturation that is associated with the rainy season, changes in water temperature and photoperiodicity (de Graaf and Janssen, 1996). In areas with two rainy seasons, this species usually has two annual reproductive peaks. Spawning starts in March just after the beginning of the first heavy rains and it is characterized by increased gonadosomatic index (GSI). Reproduction is generally completed in July or August and the GSI decreases until November. Thereafter, the oocytes gradually mature and become ripe again in March (Owiti and Dadzie, 1989; Mgbenka and Eyo, 1992; Yalçin et al., 2001).

Sexual maturity occurs at the age about one year. Males have long urogenital papilla of conical shape, whilst, females have star-shaped papilla. Before spawining, they undertake reproductive migrations from the larger water bodies to temporarily flooded shallow areas (Viveen et al., 1986; de Graaf and Janssen, 1996; Hamackova et al., 2007; Kouril et al., 2013; FAO, 2016). Spawning usually occurs at night at a water temperature above 22 °C. Prior to spawning, males compete aggressively for females with which they spawn in single pairs. In a courtship ritual the male twists around the female in U-shape. The male releases sperm as the female releases eggs and moves her caudal fin vigorously to mix the eggs and sperm, and to distribute the fertilized eggs. The adhesive eggs stick to submerged vegetation (Hecht et al., 1988; FAO, 2016). The African sharptooth catfishes have no parental care but they are highly fecund (Sydenham, 1980; Eyo and Mgbenka, 1992). One female may lay about 60000 of eggs per one kilogram of body weight. After several weeks, a new batch of eggs is developed, thus the fish may spawn several times during one season (Viveen et al., 1986; de Graaf and Janssen, 1996; Hamackova et al., 2007; FAO, 2016).

In captivity, the female of African sharptooth catfish have fully developed ovaries, producing ripe eggs throughout the entire year at temperatures above 22 °C (de Graaf and Janssen, 1996). Both sexes can be reared together in ponds, cages or recirculating aquaculture systems at temperature of 23–25 °C (Hamackova et al., 2007). Females and males sexually mature at the age of 5–7 months (Legendre et al., 1992) and 7–11 months (Van Dyk and Pieterse, 2008), respectively. In ponds, fish may spawn spontaneously within a few months of the year. However, in RAS (Recirculating Aquatic System) they can be artificially spawned throughout the year using hormonal stimulation (Viveen et al., 1986; Haylor, 1993; Hamackova et al., 2007). After Hogendoorn (1983), the minimal interval between repeated induced ovulations is approximately 6–7 weeks without loss of fecundity. Ovulation can be stimulated with carp pituitary (Hogendoorn, 1977; Hogendoorn and Vismans, 1980; de Graaf et al., 1995; Masar et al., 1998; Adamek, 2001; Brzuska, 2011; FAO, 2016), or commercial hormonal preparations (de Leew et al., 1985; Viveen et al., 1986; Kouril et al., 1992; Brzuska et al., 2004; Brzuska, 2011; FAO, 2016). Unfortunately, males do not release semen, even after hormonal treatments (Hogendoorn, 1979). Therefore, males are usually sacrificed following hormonal therapy, testes are dissected, cut into small pieces and then intra-testicular semen is obtained (Viveiros et al., 2002). Females are hand-stripped by gently pressing their abdomen from the pectoral fin towards the genital papilla and eggs are collected in a dry container. After that, the intra-testicular semen is spread over the ovulated eggs and activated by adding an equal volume of clean water of appropriate temperature (de Graaf and Janssen, 1996). The sperm are motile for 80 to 120 s (Hecht et al.,1988) and within this time they fertilize the eggs. After contact with water, the eggs become sticky and they clump to each other, but their adhesiveness can be removed with various solutions such as urea, milk, mud, or talcum (Asraf et al., 2013).

1.3. REARING OF EARLY STAGES

In natural habitats, the African sharptooth catfish eggs hatch at the overgrown temporarily flooded shallow areas after 20–60 hours post fertilization, depending on the temperature (usually more than 24 °C). The hatched larvae start to swim after 1–2 days post hatching. Then, they seek out dark places among the vegetation in the marginal areas which are usually free of predators and rich in food resources. Within 3–4 days post hatching, the larvae absorb their yolk and start to feed on plankton. Their growth is rapid, reaching 20–30 cm at one year of age. Sexual differentiation begins between 10–15 days post hatching. Within 3–4 weeks from the onset of external feeding ingestion, the arborescent suprabranchial organ starts to be functional. After several months, the juveniles and adults undergo migration to deeper waters, when the flooded marginal areas dry up as the dry season begins (Viveen et al., 1986; Hecht et al., 1988; de Graaf and Janssen, 1996; Hamackova et al., 2007; FAO, 2016).

Under hatchery conditions, the fertilized eggs of African sharptooth catfish can be spread on immersed screens (mesh size 1 mm), or on the bottom of concrete, fiberglass, and plastic tank, also they can be stuck on the roots of various plant species. There, they should be incubated at optimal stable conditions, at temperatures between 25–27 °C, level of dissolved oxygen >90%, pH 6.5–8.0, salinity 0–0.5%, water flow 1–3 $l.min^{-1}$. Generally, because of low fertilization rate (50–60%), many eggs become white (unfertilized eggs or dead embryos) during incubation. Whereas, the fertilized eggs retain transparent and green-brown colour. Newly hatched larvae should be separated from the white eggs and remnants of egg-shells, to avoid fungal infections of hatchlings and consequent high mortality. Eggs hatch after 23–27 hours at temperature of 25–27 °C. Newly hatched larvae are 4–5 mm and weigh

1.2–1.6 mg. Rearing can be in nursery ponds, flow-through, and RAS with concrete, fiberglass, or plastic tanks (volume 100–1000 l) at a stocking density of 5000–15000 larvae per one cubic meter. Within 1–2 days post hatching, the larvae start to actively swim, search for shelter and congregate in dark parts of rearing tank (Viveen et al., 1986; Britz and Hecht, 1987; de Graaf and Janssen, 1996; Hamackova et al., 2007; Kouril et al., 2013; FAO, 2016). They are photophobic so it is recommended to overshadow the tanks (Britz and Pienaar, 1992 or Appelbaum and Kamler, 2000). After 2–3 days post hatching, the larvae should be given live food (usually by *Artemia* sp.) for at least 5 days. Thereafter, they are gradually trained to a dry diet (starter food, size 0.3–0.8 mm). Their yolk is fully resosrbed within 10–26 days after hatching, depending on the water temperature. At this time, their length is 10–20 mm and weight is 15–50 mg (Viveen et al., 1986; de Graaf and Janssen, 1996; Hamackova et al., 2007; Kouril et al., 2013; FAO, 2016).

More information about rearing of African sharptooth catfish in recirculating aquaculture systems is included in Chapter 4 (Kouril et al., 2013).

1.4. FISH ONTOGENY

Generally, ontogeny is defined as a sequence of morphological and physiological changes during individual development (Fuiman, 1994; Kamler, 2002). It starts at egg fertilization and ends with the death of individual (Sladecek, 1986).

Ontogeny is divided into five periods: embryonic, larval, juvenile, adult and senescent (Balon, 1975). Main events in early life are: egg fertilization – Fe, hatching – H, first intake of external food – S, and full yolk resorption – Re. Between, Fe and S, the only source of energy is a yolk (endogenous feeding period). From S to Re, yolk and external food are used (mixed feeding period). After Re, the exogenous feeding period begins (Kamler, 2002).

Although, studies of fish ontogeny have been carried out from 19th century (Kamler, 2002), there was a long-term discussion regarding classification and terminology in fish embryonic and larval life. Therefore, some authors have attempted to summarize previously known information (Penaz, 2001; Kamler, 2002).

Currently, two different approaches to study early development of the fish exist: saltatory ontogeny and continuous ontogeny (Kamler, 2002). The saltatory classification considers that ontogeny has a sequence of separate steps. Transition between the steps is realized via a rapid threshold. During the threshold a differentiation is accelerated, while within a step morphological state is stabilized (Kryzhanovskij, 1949; Vasnetsov, 1953; Ryzhkov, 1976; Balon, 1986). However, Kamler (2002) states that this approach has at least three deficiencies: 1) morphological state does not remain unchanged within any given step in embryos and larvae, 2) besides, the transition between steps is not too rapid, because embryos and larvae often exhibit the characters of two adjacent steps, 3) moreover, the steps are not equally distributed over the time (Kamler, 2002). On the other hand, the continuous classification describes the ontogeny as an uninterrupted process (Gorodilov, 1996).

In summary, both approaches have contributed to better understanding of fish ontogeny. Nevertheless, in the present study, the early ontogeny was classified according to Kamler (2002), who created a sort of consensus between the saltatory and continuous classifications

and regards the ontogenetic advancement as a continuous process with temporary accelerations.

1.5. EARLY ONTOGENY OF AFRICAN SHARPTOOTH CATFISH

Unfertilized egg is about 1±1.3 mm in diameter. It is oval-shaped, opaque and brownish-green in colour. Spermatozoon enter into the egg through the micropyle. Both pronuclei fuse during the fertilization event. The yolk shrinks away from the membrane and egg adhesiveness develops (de Graaf and Janssen, 1996; Sule and Adikwu, 2004; Olaniyi and Omitogun, 2013).

In African sharptooth catfish, as in most of the teleosts, the developing eggs undergo meroblastic discoidal type of cleavage. This is characterized by a yolk mass (at the vegetal pole) with developig blastodisc at the animal pole, followed by mounding formation of the one-cell stage follows (Fig. 3B). The first mitotic division forms two blastomeres (Fig. 3C), followed by the second division perpendicular to the first forming four blastomeres (Fig. 3D). Division continues resulting in doubling the number of blastomers (8-cell stage, 16-cell stage and 32-cell stage, 64-cell stage, etc.) until the morula stage (Fig. 3E and 3F). Then proliferation of the blastocoele forms a dome-shaped structure enveloping major part of the yolk (blastula stage – Fig. 3G). The blastoderm expands with the overgrowth of the yolk, or the onset of epiboly. The epiboly continues with the uniform and random quasi-peristaltic wave movements. Epiblast and hypoblast form. The closure of the blastopore marks the end of this movement and the beginning of morphogenesis. Embryonal axis with the cephalic and tail buds are revealed and somites form. Somite stage (Fig. 3H) is characterized by a foul smell that continue till hatching. Occasional embryonic movements can be seen. First heartbeat occurs. The tail and head become slightly separated from the yolk. At hatching, the embryo's tail breaks through the chorion (Olaniyi and Omitogun, 2013).

At a temperature of 28.5±0.5 °C, the newly hatched larvae are characterized by uniform pigmentation and active circulatory system. Hatched larvae are translucent and 5.0±0.5 mm length (Fig. 3I). Eye pigmentation is discernible. Hatched larvae are photophobic. About one hour after hatching, blood circulation starts. Gradually, rudimentary excretory system develops and melanophores spread over the head. The oral opening is not yet developed. Notochord, heart and full yolk are obvious. Barbels are present. Second day after hatching, larvae measure 6.0±0.5 mm length. The melanophores spread towards dorsal area, the digestive system is considerably developed and yolk-sac volume is reduced. Upper and lower jaws begin to form. The alimentary canal is distinct. Blood circulation is visibly vascularized. Rudimentary caudal rays and operculum can be recognized. Third day post hatching, eye musculature and operculum are well developed. Yolk-sac is significantly reduced. Larvae are able to swim. Very few larvae start to intake exogenous feeding. At that time larvae are 8.7±0.5 mm in length (Fig. 3J). At fourth day from hatching, melanophores spread over the entire body. Larvae measure 9.3±0.5 mm. Yolk-sac is significantly reduced (Fig. 3K). The digestive system is well developed. All larvae are feeding exogenously. Urogenital orifice resumes functionality. Buccal and branchial systems are fully vascularized. Barbels are longer and segmented. Muscle folds are thicken. Caudal fin rays are visible (Olaniyi and Omitogun, 2013).

Early development of African sharptooth catfish was also studied by Legendre and Teugels (1991), Sule and Adikwu (2004), Osman et al. (2008) and Kipper et al. (2013).

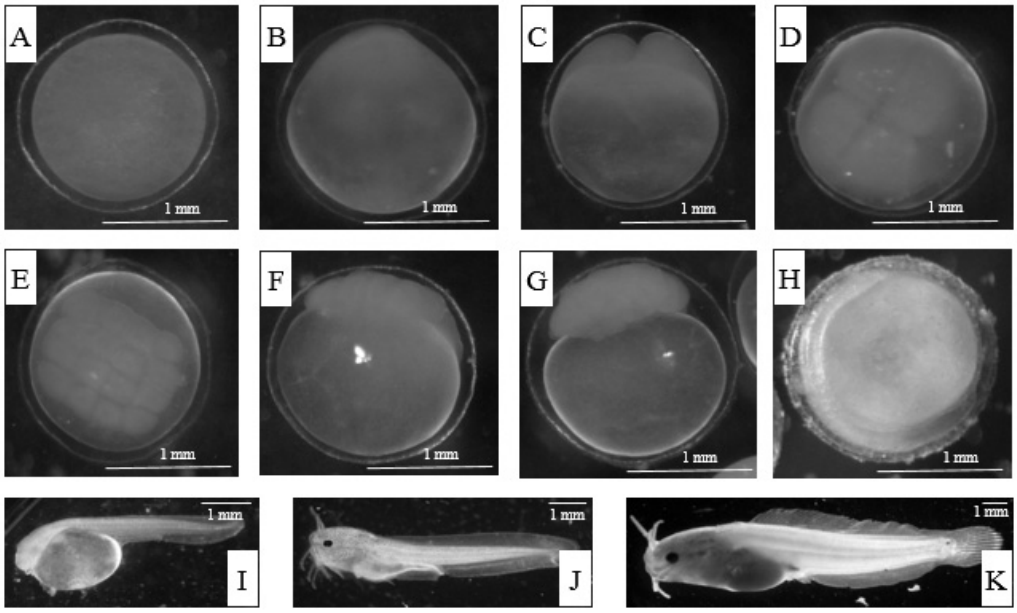

Figure 3. *Early development of African sharptooth catfish: A – fertilized egg, B – one-cell stage, C – two-cell stage, D – four-cell stage, E – sixteen-cell stage, F – morula, G – blastula, H – somite completed, I – hatched larva, J – larva at the onset of food ingestion, K – externally fed larva at full yolk resorption (photo by M. Prokesova).*

1.6. FACTORS INFLUENCING FISH ONTOGENY

Fish ontogeny is influenced by many endogenous and exogenous factors, as well as by interactions between these factors. Endogenous factors are egg size and parental effect, whilst, exogenous factors are classified into abiotic (temperature, oxygen, salinity, pH, light, dissolved biotic compounds, anthropogenic factors, magnetic fields, husbandry in aquaculture) and biotic factors (predation, parasitism, diseases, competition) (Wooton, 1990; Boeuf and Le Bail, 1999; Kamler, 2002). In aquaculture, proper management of those factors is key to successful commercial production. Effects of selected abiotic factors (temperature and light), which were studied in present work, are described below (Chapter 1.6.1. and 1.6.2.).

1.6.1. Temperature

The temperature is considered as one of the most important abiotic factors influencing fish ontogeny (Bauer, 1962; Chubb, 1982; Kamler, 1992, 2002; Kupren et al., 2008). Within a zone of thermal tolerance (see description below), it is a factor, which can cause acceleration or deceleration of ontogenetic rate by higher or lower water temperatures, respectively. Whereas, out of the zone of thermal tolerance, temperature is a lethal factor (Brett, 1979; Kamler, 2002; Korwin-Kossakowski, 2008). Generally, the thermal tolerance of fish fluctuates through their life time (Motani and Wainwright, 2015). Thermal limits are narrower for early life stages (embryos and larvae) than later developmental stages (juveniles and adults) (Kupren et al., 2010; Souchon and Tissot, 2012). However, the thermal tolerance of fishes can be affected by their thermal history or acclimation temperature (Kokurewicz, 1971; Kujawa et

al., 1997). In general, data about the thermal preferences of fish species are very important for their culture, but also in the context of the current global warming of the water due to climatic changes (Daufresne and Boet, 2007; Motani and Wainwright, 2015).

Four temperature zones are distinguished in fishes (Souchon and Tissot, 2012):

1) Thermal tolerance: the temperature range where the fishes can live without developmental and growth variations (Lapkin et al., 1981).
2) Thermal resistance: the higher and lower temperature ranges outside the zone of thermal tolerance (Lapkin et al., 1981) where the fishes may live for a limited, specific time period (Wieser, 1991). Some authors (as Kupren et al., 2011) use the term sublethal temperatures which is characterised by an evident decrease in survival rate.
3) Lethal temperatures: the temperatures where the fish die (Lapkin et al., 1981).
4) Optimal temperatures: the temperature range within the zone of thermal tolerance (Pavlov, 2007). It can vary with respect to development, growth, survival, and metabolic rate of species (Kamler, 2002; Pavlov, 2007). For example, Kupren et al. (2011) state that the optimal thermal range provides the best developed embryos and the highest survival rate until the hatching of larvae. Other authors (Penaz et al., 1983) specify this value of survival rate as a thermal range where at least 60% of individuals survive. Whilst, other authors (Brett, 1971 and Kellogg and Gift, 1983) recognize the optimum temperature for growth which coincides with a preferred temperature in a vertically or horizontally arranged thermal gradient in tank (Tsuchida and Fukataki, 1991). Besides, Kokurewicz (1969) and Kamler (1992) add that the optimal temperature range for incubation could be determined from the data about thermal tolerance of developing eggs together with information about hatched individuals (such as abnormalities, body size etc.).

Generally, fishes can be classified according to the temperature requirements. Stenothermal and eurythermal are the species which tolerate narrow or wide thermal range, respectively (Wieser, 1991). Fishes also can be divided into coldwater (tolerating low temperatures), warmwater (tolerating high temperatures) and mesothermal (tolerating mid temperatures) species (Wieser, 1991; Kamler, 2002).

Effect of temperature (t, °C) on ontogeny can be expressed by changes in time (τ, days) from fertilization to any ontogenetic state. Overall, six models quantify this relationship: exponential, power law, Belehradek´s, Leiner´s, polynomial and linear model (summarized by Kamler, 2002). Nevertheless, only the linear model ($V = a + bt$) describes the ontogenetic rate well over the whole zone of thermal tolerance and it seems suitable to compare temperature requirements between species. Moreover, three biologically meaningful parameters can be derived from the model. *Temperature of biological zero* ($t_0 = -a / b$) at which the ontogeny is theoretically arrested, an *effective temperature* ($t_{eff} = t - t_0$), and number of *effective day-degrees* above the *temperature of biological zero* [$D°_{eff} = \tau (t - t_0) = b^{-1}$]. Then, the time from t_0 to any ontogenetic event can be recalculated [$\tau = D°_{eff} / (t - t_0)$]. Effective day-degrees are temperature independent measure of ontogenetic advancement. In warmwater species, the high t_0 is accompanied by low $D°_{eff}$ (Kamler, 2002).

The effect of water temperature on African sharptooth catfish has been studied in relation to growth of larvae and juveniles (Britz and Hecht, 1987), onset of air breathing and development of accessory breathing organs (Haylor and Oyegunwa, 1993), optimal temperature range, effective temperature, estimation of threshold and lethal temperature, incubation and hatching time, duration between first feeding, yolk absorption and point-

of-no-return, developmental, growth and metabolic rate, matter composition (Legendre and Teugels, 1991; Kamler et al., 1994; Haylor and Mollah, 1995;). However, the temperature effect on the early development of African sharptooth catfish has not been investigated in detail and the zone of thermal tolerance with the optimal thermal range should be more specified.

1.6.2. Light

Light includes its intensity, spectrum (wavelength) and periodicity (photoperiod) (Boeuf and Le Bail, 1999; Lee et al., 2014). Light influences the development of organisms from early life to adult stages (Mangor-Jensen and Waiwood, 1995; Downing and Litvak, 2002). Development of visual system of larvae is species-specific (Politis et al., 2014). Moreover, light receptivity varies between the developmental stages, as the number of cones in the retina increases during the early ontogeny (Blaxter, 1969, 1975).

According to the literature, larvae of fishes can be classified depending on light requirements into three groups. Some larvae survive better when they are reared under certain photoperiod regimes (*Solea senegalensis* – Canavate et al., 2006; *Argyrosomus regius* – Valles and Estevez, 2013). Other fish species develop better in complete darkness (*Hippoglossus hippoglossus* – Bolla and Holmefjord, 1988; *Sparus aurata* – Sahin et al., 2001), or under constant lightening (*Melanogrammus aeglefinus* – Downing and Litvak, 1999; *Gadus morhua* – Puvanendran and Brown, 2002).

Generally, light can affect all aspects of fish biology, including stress responses (Billard et al., 1981), activity rhythms (Schwassmann, 1971, Boujard and Leatherland, 1992), metabolism (Boeuf and Le Bail, 1999), feeding behaviour (Tandler and Helps, 1985) and gonad development (Baggerman, 1980). Light may influence development of early fish stages (Kamler, 2002; Andrew et al., 2009; Blanco-Vives et al., 2010; Villamizar et al., 2011). It can influence their incubation period (Downing and Litvak, 2002), hatching rate (Helvik and Walther, 1992; Ellis et al., 1997), yolk utilization efficiency (Watanabe, 1998), size of larvae (Ellis et al., 1997; Watanabe, 1998; Downing and Litvak, 2002), first feeding (Downing and Litvak, 2001; Carton, 2005), survival rate (Gulbrandsen et al., 1996; Hart et al., 1996; Ellis et al., 1997), and occurrence of deformities (Bolla and Holmefjord, 1988; Politis et al., 2014). All in all, the fish development can be accelerated, decelerated or not influenced by light. However, the response to light conditions is related to maternal effect, environmental adaptations, age and species of fish (Zhukinskij, 1986; Boeuf and Le Bail, 1999; Kamler, 2002; Politis et al., 2014).

Above all, influence of light periodicity, also continuous lightening or darkness on African sharptooth catfish early stages have been investigated. The studies were focused mostly on hatching (Appelbaum and Kamler, 2000; Mino et al., 2008), survival (Appelbaum and Kamler, 2000; Mino et al., 2008), initiation of exogenous feeding (Appelbaum and Kamler, 2000; Mukai and Lim, 2011), growth (Britz and Pienaar, 1992; Appelbaum and Kamler, 2000; Mino et al., 2008), behaviour and cannibalism (Britz and Pienaar, 1992; Appelbaum and Kamler, 2000; Adamek et al., 2011; Mukai et al., 2013). Nevertheless, there is lack of information about effect of light intensity and spectrum on African sharptooth catfish early life stages, as well as the juveniles.

1.7. AIMS OF PRESENT PH.D. THESIS

The aim of the present thesis was to specify the effect of abiotic factors (thermal and light conditions) on the early ontogeny (from egg fertilization until the full yolk absorption) of African sharptooth catfish with subsequent implementation of the results into commercial production, where additional revision of the currently existing technologies (including the spawning induction, gamete management, intensive rearing) were performed and collected in the form of handbook including new detailed production technology. The thesis was divided into three thematic parts constituting specific aims:

1. In the first study, the aim was to describe the effect of water temperature (range: 17.4–35.6 °C), as a meaningful abiotic factor, during early ontogeny from egg fertilization until the full yolk absorption in African sharptooth catfish (Chapter 2 – Prokesova et al., 2015). Especially, the study was focused on: incubation period, process of hatching, yolk-sac absorption, intake of exogenous feeding, growth and survival rate.

2. In the second study, the aim was to assess, the effect of light intensity (range from <0.1 to >5000 Lx) during early ontogeny from egg fertilization until the full yolk absorption in African sharptooth catfish (Chapter 3 – Prokesova et al., 2016). In particular, the study was especially focused on: incubation period, process of hatching, yolk-sac absorption, intake of exogenous feeding, growth and survival rate.

3. The aim of the third study was to prepare detailed technology handbook about rearing of African sharptooth catfish (Chapter 4 – Kouril et al., 2013) available to fish farmers and public audience. Study includes new information about rearing, artificial reproduction, effect of temperature on storing of eggs, effect of water contamination on closing time of egg micropyle, effect of temperature on egg incubation and larval rearing, results of feeding and sensory experiments. Part of these results (effect of temperature on egg incubation and larval rearing) is included in the present dissertation thesis.

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CHAPTER 2

EFFECT OF WATER TEMPERATURE ON EARLY LIFE HISTORY OF AFRICAN SHARPTOOTH CATFISH, *CLARIAS GARIEPINUS* (BURCHELL, 1822)

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Effect of water temperature on early life history of African sharp-tooth catfish, *Clarias gariepinus* (Burchell, 1822)

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Summary

Early life history of *Clarias gariepinus* from egg fertilization to full yolk sac resorption was studied at 33 temperatures within the thermal range 17.4–35.6°C. The viable temperature range for embryonic development; temperature at which ontogeny is hypothetically arrested (15.4°C); viable temperature range for larval development; and the number of effective day-degrees for the embryonic and larval periods were determined. The early life history of *C. gariepinus* was found to be temperature-dependent in terms of the onset of key ontogenetic events, developmental rate, survival rate, and size of larvae. The length of the incubation period, hatching period, size of larvae at hatching, time to first intake of exogenous food, and time to full yolk sac resorption with and without exogenous feed supply, were inversely proportional to the temperature within the optimal temperature range. In terms of survival, the zone of thermal tolerance for early life history of *C. gariepinus* ranged from 18.9 to 33.2°C with the thermal optimum from 22.9 to 30.3°C, typical for thermophilous species. Temperature ranges of 20.6–22.9°C and 30.2–33.2°C were found to be suboptimal. Temperatures below 17.5 and above 35.1°C may be considered lethal during the embryonic period, and those below 18.9 and above 33.2°C are lethal during the larval period.

Introduction

The African sharp-tooth catfish, *Clarias gariepinus* (Burchell, 1822), is native to subtropical and tropical fresh waters of Africa and Asia (Viveen et al., 1986; Appelbaum and Kamler, 2000; Kůrka et al., 2000) and has been introduced into many countries due to biological characteristics allowing it to be successfully reared at stocking densities of 300–400 kg m^{-3} and in water with low oxygen level, high ammonia concentration, and organic pollution. *Clarias gariepinus* is resistant to diseases and parasites and characterized by rapid growth, muscle with high protein and low lipid content, absence of intramuscular bones, and early reproduction. Spawning can be induced in females several times per year. Hence, *C. gariepinus* is well suited to intensive aquaculture (Appelbaum and Kamler, 2000; Kouřil et al., 2012). The worldwide commercial production of *C. gariepinus* has increased exponentially over the past three decades, from ca. 50 tonnes (t) in 1980 to ca. 191 000 t in 2010 (FAO, 2012).

Embryonic and larval development is a critical phase in fish life history, characterized by high sensitivity to external environmental conditions and often manifested by high mortality of early ontogenetic stages. The study of early fish ontogeny is crucial to understanding species biology and ecology and provides valuable knowledge for successful rearing (Verreth et al., 1992; Meijide and Guerrero, 2000; Koumoundouros et al., 2001; Borcato et al., 2004).

Ontogeny may be affected by various endogenous (parental effect, egg size) and exogenous (temperature, oxygen concentration, pH, salinity, water currents) factors. Water temperature is of considerable importance, affecting fish, as poikilothermic organisms, with respect to development, growth, metabolism, and movement. The understanding of the effect of temperature on early ontogeny is essential for successful rearing of fish (Kamler, 2002; Drozd, 2011). Although studies of the temperature effect on the early development of *C. gariepinus* have been conducted (Kamler et al., 1994; Haylor and Mollah, 1995), thus far the topic has not been investigated in detail, and the viable temperature range for *C. gariepinus* has not been determined.

In the present work, detailed knowledge thermal dependence of early development, survival, and growth of *C. gariepinus* were carried out for better understanding of the biology of this species.

Materials and methods

Materials

Thirty (5 stripping^{-1}) females and 18 (3 stripping^{-1}) males (1–3 kg body weight) *C. gariepinus* were kept in recirculation aquaculture systems at 25°C; pH 7–8; 40–60% O_2; 12:12 L:D. Generally, six induced spawnings were conducted. Females were anaesthetized, and ovulation was induced by Ovopel (20 μg GnRHa + 2 mg metoclopromide; Agrofish Ltd., Hungary) at 1 pellet kg^{-1} of body weight. Eggs were stripped into dry bowls. Males were manually killed, dissected and sperm was obtained from testes for immediate use for dry fertilization. Talc was applied to fertilized eggs at 25 g L^{-1} of water for 1 h to prevent sticking.

Table 1
Temperature ranges (mean ± SE; °C) and feeding modes tested during experiments (1st–6th).

Spawning	Feeding mode	Incubation temperature (mean ± SE; °C)						
1st	Feeding	17.5 ± 0.09	21.5 ± 0.05	24.0 ± 0.04	27.3 ± 0.29	30.3 ± 0.24	33.2 ± 0.25	35.6 ± 0.45
2nd	Feeding	25.5 ± 0.25	27.1 ± 0.19	28.6 ± 0.22	30.2 ± 0.26	31.6 ± 0.14	33.2 ± 0.11	
3rd	Unfeeding	17.4 ± 0.10	18.9 ± 0.08	20.6 ± 0.21	22.9 ± 0.03	25.2 ± 0.10		
4th	Unfeeding	27.2 ± 0.12	29.1 ± 0.17	31.5 ± 0.14	33.7 ± 0.06	35.1 ± 0.11		
5th	Unfeeding	27.2 ± 0.04	29.3 ± 0.11	31.4 ± 0.04	33.6 ± 0.03	35.2 ± 0.17		
6th	Unfeeding	23.2 ± 0.15	25.4 ± 0.27	27.1 ± 0.07	29.3 ± 0.13	31.2 ± 0.04		

Rearing conditions

Nylon net with approximately 30 000 fertilized eggs and four 1-L glass aquaria with 100 fertilized eggs (in four replicates) were incubated in temperate bath units with a flow-through water system at 33 stabilized temperatures (temperature range 17.4–35.6°C; pH 7–8.5; 70–100% O_2; 12:12 L:D). Individuals reared in nets were used for examination at selected critical ontogenetic events: fertilization-Fe, hatching-H, first intake of exogenous food-S, and yolk sac resorption-Re. Individuals reared in aquaria were monitored for survival.

Following hatching, the units were covered with black foil as recommended by Appelbaum and Kamler (2000). For finding out of possible effect of feeding mode (with/without exogenous food supply) on various morphometric characteristics as well as time aspects, after shift to mixogenous feeding larvae originating from spawning 1, 2 were fed every three hours on live brine shrimp nauplii, *Artemia salina*, while those originating from spawning 3, 4, 5, 6 were nonfed (they were starving). Dead larvae were removed daily and numbers recorded. Larvae were reared in these conditions (Table 1) up to 6 days after complete yolk sac resorption.

Sampling

One hundered and fifty unfertilized eggs were photographed by binocular magnifying glass (Olympus, SZX 9, Tokyo, Japan) with digital camera (Olympus Camedia C5060UW). Width and height were measured using image analysis software (MicroImage version 3.0.1. for Windows) on digital photographs. To determine of mean wet weight (WW) of one egg, 50 eggs (in three repetitions) were weighed on an analytical balance. To determine the mean dry weight of one egg (WD), 150 eggs were dried in an oven at 60°C for 12 h and weighed on an analytical balance.

Eggs incubated in glass aquaria were monitored every two hours. This interval was reduced to 30 min when the first eggs hatched. Hatching time of 5, 50, 75 and 95% of individuals (H_5, H_{50}, H_{75}, H_{95}, respectively) was calculated using the cumulative percentage of hatched eggs (P), which was linearized by transfer from percent to logit and plotted against the logarithm of time.

$$\text{logit} = \log_{10}[(0.01 \cdot P/(1 - 0.01 \cdot P)]$$

The relationship between developmental rate and temperature during the embryonic period was defined using linear model of Kamler (2002).

$$V = a + b \cdot t\,(\text{days}^{-1})$$

Two additional parameters were derived from this model: threshold temperature at which the ontogeny is hypothetically arrested (biological zero, t_0)

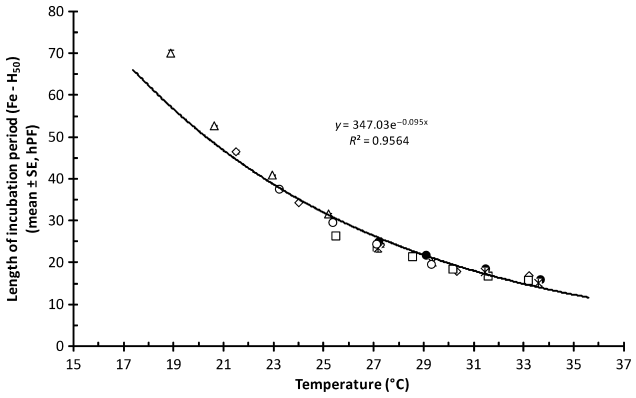

Fig. 1. Dependence of incubation period (Fe–H_{50}; mean ± SE; hPF) on water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = a \cdot e^{-bx}$. ◊, 1st spawning; □, 2nd spawning; △, 3rd spawning; ●, 4th spawning; ✳, 5th spawning; ○, 6th spawning; R^2, correlation coefficient

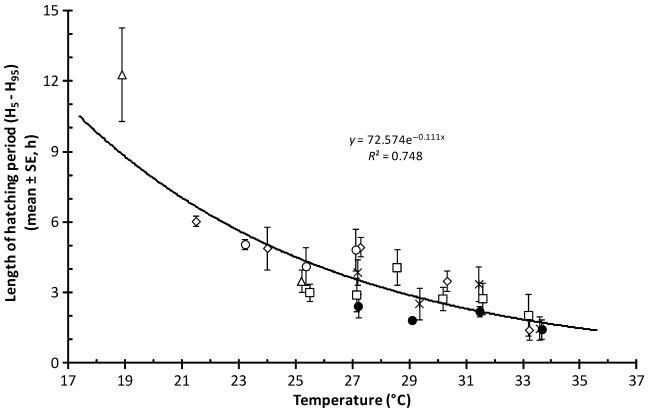

Fig. 2. Length of hatching period (H_5–H_{95}; mean ± SE; h) with water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = a \cdot e^{-b \cdot x}$. ◇, 1st spawning; □, 2nd spawning; △, 3rd spawning; ●, 4th spawning; ⁎, 5th spawning; ○, 6th spawning; R^2, correlation coefficient

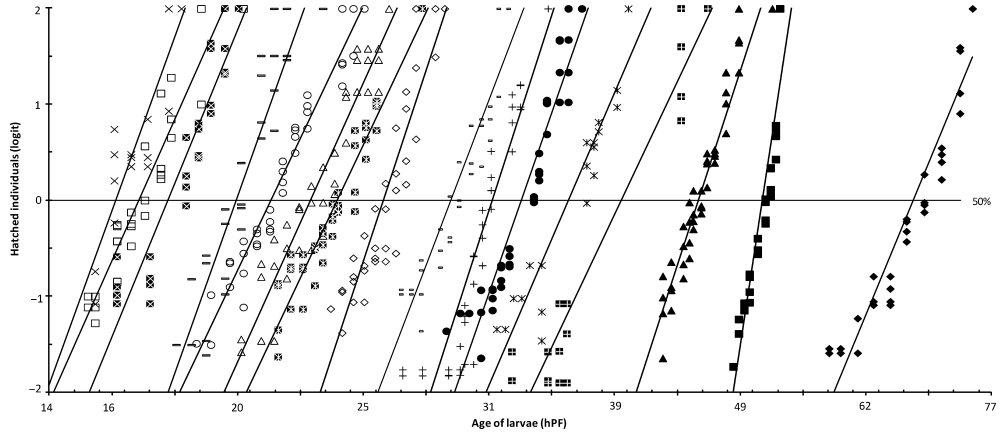

Fig. 3. Cumulative percentage of hatched individuals (converted to logits and plotted against the logarithm of time, hPF) in relation to incubation temperature (°C) in *Clarias gariepinus*. Hatching time of 50% of eggs (H_{50}) corresponds to logit 0. ♦, 18.88°C; ■, 20.62°C; ▲, 21.49°C; ▪, 22.94°C; ⁎, 23.22°C; ●, 24.00°C; +, 25.19°C; –, 25.36°C; ◇, 25.48°C; △, 27.17°C; ▩, 27.27°C; ○, 28.55 °C; –, 29.34°C; ▩, 30.32°C; □, 31.57°C; ×, 33.18°C

$$t_0 = -a/b,$$

and a number of effective day-degrees (number of degrees above biological zero; D°_{eff}).

$$D^{\circ}_{\text{eff}} = \tau \cdot (t - t_0) = b^{-1}$$

The lowest viable temperature (temperature at which ontogeny is still in progress; t_{lowest}) was calculated according to the mathematical model proposed by Kamler (2002).

$$t_{\text{lowest}} = 1.34 + 0.929 \cdot t_0$$

For any critical ontogenetic event (H_{50}, $S_{50,}$ Re_{50}), 30 individuals were sampled. Larvae were anaesthetized in clove oil (0.05 ml L^{-1}; Dr. Kulich, Czech Republic), weighed on an analytical balance to determine the mean WW of a single larva, photographed by the binocular microscope fitted by digital camera, dried at 60°C for 12 h, and weighed on an analytical balance to determine the mean WD of a single larva. Digital photographic images were measured using image analysis software to record total length (TL), standard length (SL), yolk sac length (YSL), and yolk sac depth (YSD). Yolk sac volume (YSV) was calculated according to the formula for an elongated spheroid (Blaxter and Hempel, 1963).

$$YSV = [\pi/6] \cdot YSL \cdot YSD^2$$

Selected approach of early ontogeny classification

Early ontogeny was classified according to Kamler (2002). The time of egg fertilization (Fe) was considered as the beginning of the embryonic period. The time at which 50% of eggs are hatched (H_{50}) constitutes the end of the embry-

Table 2
Clarias gariepinus regression equations and correlation coefficients for mathematical expression of cumulative percentage of hatched eggs (expressed in logits plotted against logarithm of time, hPF) relative to water temperature (°C), (see Fig. 3).

T (°C)	$y = ax + b$	R^2
18.88(3)	31.64*x*−58.15	0.91
20.62(3)	86.24*x*−148.15	0.86
21.49(1)	40.35*x*−67.25	0.93
22.94(3)	27.66*x*−44.41	0.94
23.22(6)	30.48*x*−47.64	0.89
24.00(1)	37.59*x*−57.36	0.91
25.19(3)	40.69*x*−61.14	0.87
25.36(6)	34.40*x*−50.55	0.88
25.48(2)	39.75*x*−56.29	0.82
27.17(5)	29.02*x*−39.41	0.91
27.27(1)	27.65*x*−38.13	0.94
28.55(2)	27.48*x*−36.45	0.95
29.34(5)	36.83*x*−47.84	0.87
30.32(1)	31.96*x*−39.76	0.89
31.57(2)	29.43*x*−35.94	0.89
33.18(2)	36.20*x*−43.55	0.78

T, temperature; y = ax + b, regression equation; R^2, correlation coefficient.

onic period and the beginning of the larval period. The larval period concluded at yolk sac resorption in 50% of larvae (Re_{50}).

Data processing

Data were evaluated for statistically significant differences among temperatures using one-way analysis of variance (ANOVA) with subsequent Tukey HSD test (Statistica 10; Stat Soft, Inc., Tulsa, OK). Parameters analyzed were egg width, height, and wet weight; length of incubation period; length of hatching period (H_5-H_{95}); size of larvae (TL, SL) at H_{50}, S_{50}, and Re_{50}; YSV of larvae at H_{50} and S_{50}; WW of larvae at H_{50}; survival at Fe-H_{50}, H_{50}-Re_{50}, and Fe-Re_{50} intervals.

Statistical evaluation was not possible for dry weight of egg and larva at H_{50}, lengths at the H_{50}-S_{50} and H_{50}-Re_{50} periods, or WW and WD of larvae at H_{50} and Re_{50}, due to lack of data. In most cases, the number of available larvae was insufficient for conducting three or more replications. In this case, only one or two measurements were conducted. Therefore, at least 30 individuals were used for each evaluation of the average of these parameters.

Results

Egg size

Width, height and WW of spawned eggs significantly varied among the spawnings [width: $F_{(3,599)}$=856.12, $P < 10^{-4}$, height: $F_{(3,599)}$=548.70, $P < 10^{-4}$, and WW: $F_{(3,11)}$=12.08, $P < 10^{-3}$]. Egg diameter (measured as width and height) ranged from 1.27 to 1.72 mm and WW from 1.02 to 1.51 mg. Mean WD of eggs was 0.30 mg per egg.

Incubation period

The length of the incubation period (Fe–H_{50}) was significantly inversely associated with water temperature [$F_{(22,69)}$=2618.89, $P < 10^{-4}$] (Fig. 1). The longest incubation period, 70.08 hours post-fertilization (hPF) on average, was found at the lowest temperature (18.9°C), and the shortest incubation period (15.13 hPF) was recorded at the highest temperature (33.6°C). No eggs hatched at temperatures of 17.3, 17.5, 35.1, 35.2 and 35.6°C. At 17.5°C embryo movement was observed until 75.42 hPF; no eggs survived thereafter.

Hatching period

Length of the hatching period (H_5–H_{95}) was significantly inversely correlated with water temperatures [$F_{(22,69)}$=25.62, $P < 10^{-4}$]. Thus the synchronization of hatching increased with increasing temperature (Fig. 2). The longest hatching period (12.29 h) was observed at the lowest temperature (18.9°C), and the shortest hatching period (1.44 h) was found at the highest temperature (33.7°C) (Fig. 3 and Table 2).

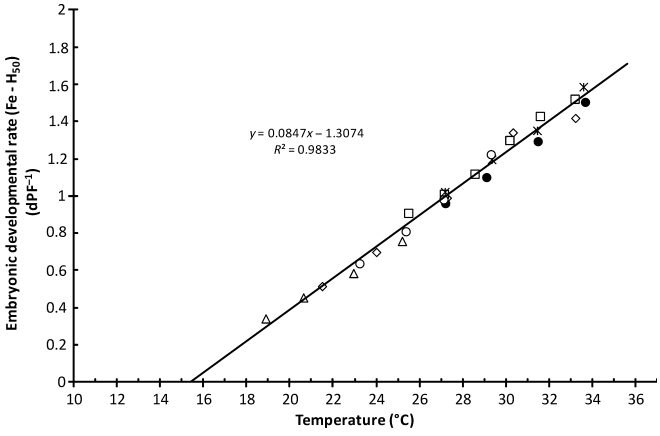

Fig. 4. Relationship of embryonic developmental rate (Fe–H_{50}; dPF^{-1}) to water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = ax + b$. ◇, 1st spawning; □, 2nd spawning; △, 3rd spawning; ●, 4th spawning; ✳, 5th spawning; ○, 6th spawning; R^2, correlation coefficient

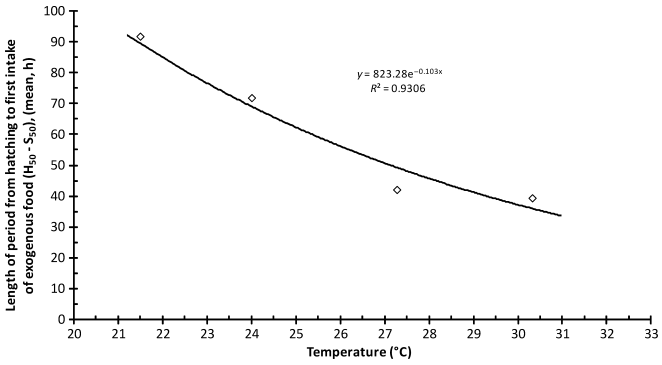

Fig. 5. Time from hatching to first intake of exogenous food (H_{50}–S_{50}; h) with water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = a \cdot e^{-bx}$. ◇, 1st spawning; R^2, correlation coefficient

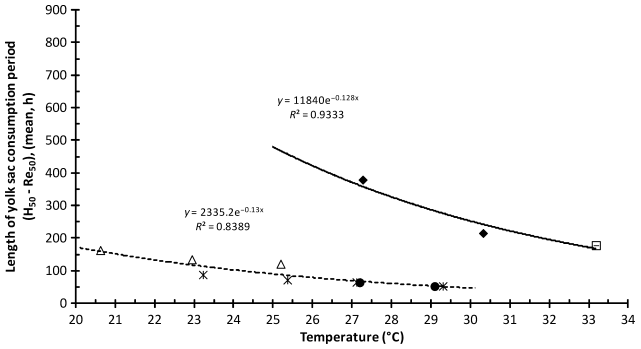

Fig. 6. Length of yolk sac consumption period (H_{50}–Re_{50}; expressed as mean; h) with water temperature (°C) and method of rearing (with/without exogenous feeding) in *Clarias gariepinus*. Data fitted by the equation $y = a \cdot e^{-bx}$. Rearing with exogenous feeding (solid line): ♦, 1st spawning; □, 2nd spawning. Rearing without exogenous feeding (dashed line): △, 3rd spawning; ●, 4th spawning; ✳, 6th spawning; R^2, correlation coefficient

Developmental rate

Embryonic developmental rate (Fe–H_{50}) increased with increasing water temperature (Fig. 4). Using the linear relationship between embryonic developmental rate and water temperature, it was estimated that the embryonic development of *C. gariepinus* is hypothetically arrested at 15.4°C (t_0), and the lowest viable temperature (t_{lowest}) is 15.7°C. Using the same relationship, it was found that the hatching of *C. gariepinus* larvae occurs after ca. 12 effective day-degrees (D°_{eff} = 11.81).

First intake of exogenous food

The average length of time from H_{50} to the first intake of exogenous food (S_{50}) decreased with increasing water temperature (Fig. 5). The longest H_{50}-S_{50} period (91.83 h) was recorded at 21.5°C, and the shortest period (39.42 h) was observed at 30.3°C.

Yolk sac consumption

The average period of yolk sac consumption (H_{50}-Re_{50}) decreased with increasing water temperature (Fig. 6). The H_{50}-Re_{50} period ranged from 178.40 to 379.27 h with exogenous feeding (spawning 1, 2) and 52.79–163.29 h without exogenous feeding (spawning 3, 4, 6).

Growth and morphometry at hatching

Larvae of *C. gariepinus* significantly differed in their TL and SL in tested water temperatures [TL: $F_{(16,382)}$=58.92, $P < 10^{-4}$; SL: $F_{(16,382)}$=58.67, $P < 10^{-4}$] at hatching (H_{50}). The largest larvae (both TL and SL) were found within the range of 21.5–31.5°C (Fig. 7). Beyond this range, TL and SL considerably decreased.

The yolk sac volume (YSV) of newly hatched larvae (at H_{50}) did not significantly differed with water temperature [$F_{(16,382)}$=50.77, $P < 10^{-4}$] (Fig. 8).

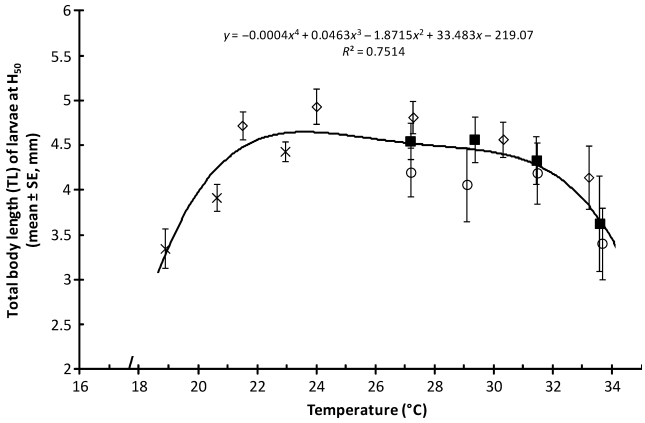

Fig. 7. Total length (TL) of larvae at H_{50} (mean ± SE, mm) with water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = ax^4 + bx^3 + cx^2 + dx + e$. ◊, 1st spawning; □, 3rd spawning; ○, 4th spawning; ■, 5th spawning; R^2, correlation coefficient

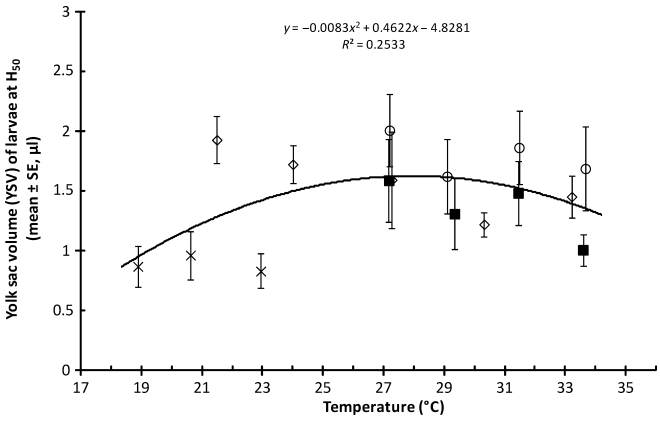

Fig. 8. Yolk sac volume (YSV) of larvae at H_{50} (mean ± SE, μl) with water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = ax^2 + bx + c$. ◊, 1st spawning; ✳, 3rd spawning; ○, 4th spawning; □, 5th spawning; R^2, correlation coefficient

Wet body weight of hatched larvae significantly differed among water temperatures [$F_{(14,25)}$=4.89, P < 10^{-3}], reaching the highest values at a range of 21.5–31.5°C. Outside of these limits the WW of hatched larvae decreased (Fig. 9).

The average individual WD of larvae reached similar values in the temperature range of 21.5 to 30.3°C. An exception was at 33.2°C, when significantly lower average WD of hatched larvae was observed (Fig. 10).

Growth and morphometry at the first intake of exogenous food

Water temperature was significantly negatively correlated with both TL and SL of larvae at the intake of first exogenous food particles in 50% of individuals (S_{50}) [TL: $F_{(3,68)}$=18.00, P < 10^{-4}; SL: $F_{(3,68)}$=23.26, P < 10^{-4}] (Fig. 11).

Yolk sac volume of larvae at S_{50} was significantly positively associated with water temperature [$F_{(3,68)}$=7.26, P < 10^{-4}] (Fig. 12).

The average individual WW of larvae at S_{50} increased with water temperature increases from 24.0 to 30.3°C (Fig. 13).

The average individual dry weight of larvae at S_{50} increased with water temperature increases from 21.5 to 27.3°C. Dry weight subsequently decreased at temperature increases from 27.3 to 30.3°C (Fig. 14).

Growth and morphometry at complete yolk sac depletion

Larvae reared without an exogenous feeding supply did not significantly differ in their TL and SL among temperatures within the thermal range of 22.9–29.1°C [$F_{(5,190)}$=7.37, P < 10^{-5}] after full yolk sac depletion (Fig. 15). However, both TL and SL significantly differed at the lowest tested temperature of 20.6°C [TL: $F_{(5,162)}$ = 4.12, P < 10^{-2}; SL: $F_{(5,162)}$=4.72, P < 10^{-2}].

The size of larvae (TL and SL) reared with an exogenous feeding supply was significantly negatively associated with

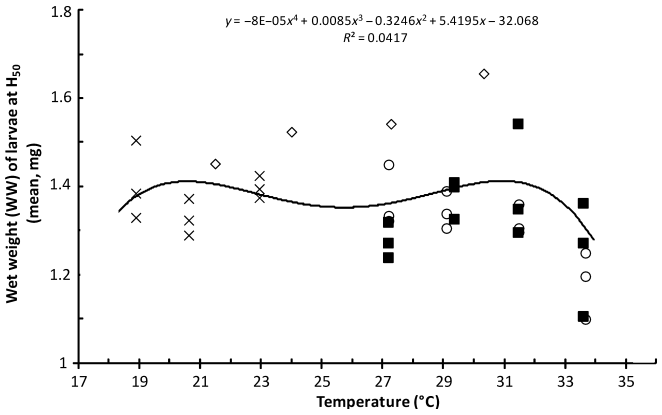

Fig. 9. Wet weight of larvae (WW) at H_{50}; (mean, mg) with water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = ax^4 + bx^3 + cx^3 + dx + e$. ◇, 1st spawning; □, 3rd spawning; ○, 4th spawning; ■, 5th spawning; R^2, correlation coefficient

water temperature at Re_{50} [$F_{(2,77)}$=106.10, $P < 10^{-3}$] (Fig. 15).

At similar water temperatures, TL and SL of larvae receiving exogenous food reached approximately twice the values of unfed larvae.

The average individual WW and WD at Re_{50} reached in larvae unfed with exogenous food similar average values across temperatures.

However, the average WW and WD individual weight at Re_{50} in larvae fed with exogenous food decreased with increasing temperature (Fig. 16).

Both the average WW and WD individual weight of fed larvae reached higher values than in unfed larvae at Re_{50}.

Survival

Survival rate of individuals significantly differed with water temperature [$F_{(17,54)}$=80.76, $P < 10^{-4}$] during the embryonic period (Fe-H_{50}). The survival rate ranged from 0 to 94%, depending on the water temperature during the embryonic period. The highest values of survival were observed at temperatures in the range of 20.6–30.3°C. All fertilized eggs died at the lowest temperatures (17.4 and 17.5°C) as well as at the highest temperatures (35.2 and 35.6°C) (Fig. 17). At comparable water temperatures, the highest survival rate was obtained at the first spawning compared to the 2nd, 3rd, and 5th spawning (Fig. 17).

The survival rate of individuals during the larval period (H_{50}–Re_{50}) significantly differed with water temperature [$F_{(9,30)}$=7.89, $P < 10^{-4}$]. Survival during the larval period ranged from 0 to 57%. The highest values were achieved at the thermal range of 22.9–30.3°C. All larvae reared at temperature below 18.9 or 33.2°C (and higher temperatures too) died (Fig. 18). At these temperatures a large number of malformed individuals were also observed.

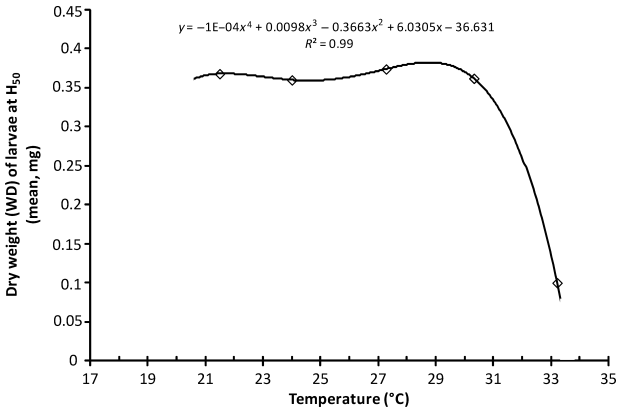

Fig. 10. Dry weight larvae (WD) at H_{50} (mean; mg) with water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = ax^4 + bx^3 + cx^3 + dx + e$. ◇, 1st spawning; R^2, correlation coefficient

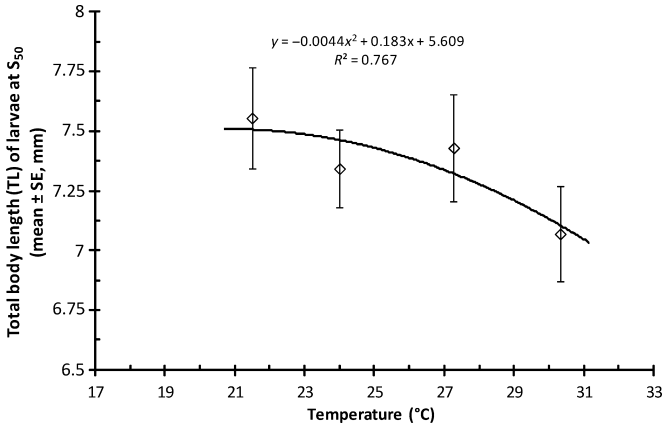

Fig. 11. Total length (TL) of larvae at S_{50} (mean ± SE, mm) with water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = ax^2 + bx + c$. ◇, 1st spawning; R^2, correlation coefficient

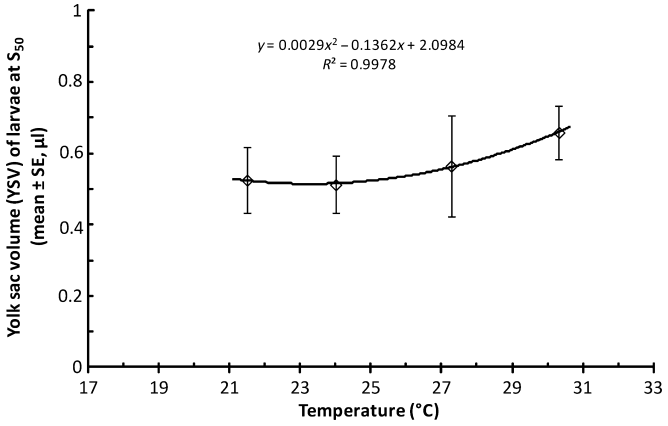

Fig. 12. Yolk sac volume (YSV) of larvae at S_{50} (mean ± SE, µl) with water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = ax^2 + bx + c$. ◇, 1st spawning; R^2, correlation coefficient

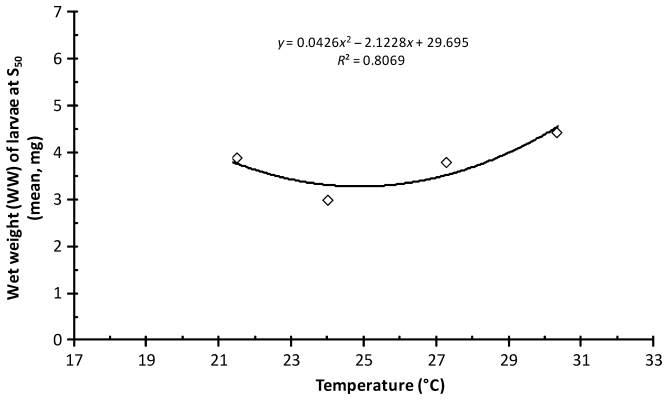

Fig. 13. Wet weight of larvae (WW) at S_{50} (mean, mg) with water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = ax^2 + bx + c$. ◇, 1st spawning; R^2, correlation coefficient

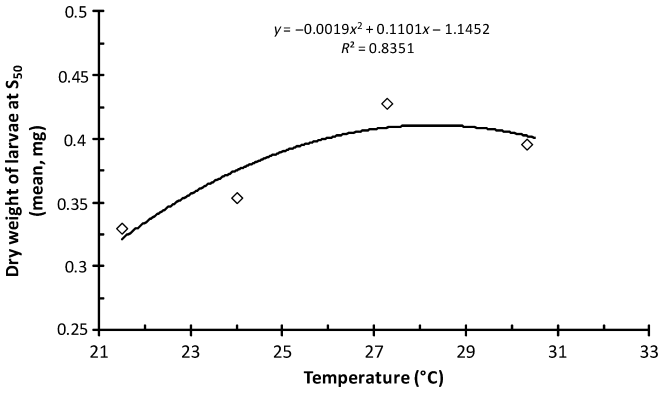

Fig. 14. Dry weight of larvae (WD) at S_{50} (mean; mg) with water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = ax^2 + bx + c$. ◊, 1st spawning; R^2, correlation coefficient

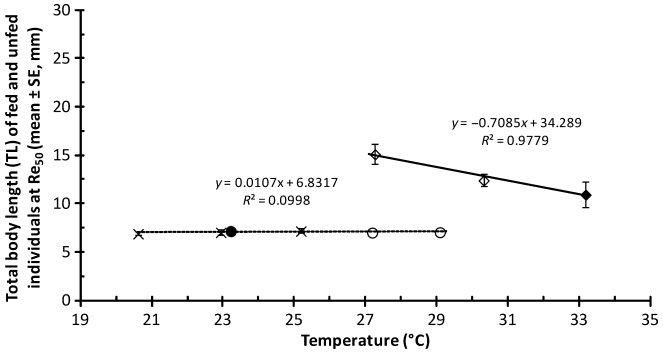

Fig. 15. Total length (TL) of fed (1st and 2nd spawnings) and unfed (3rd, 4th, and 6th spawnings) larvae with water temperature (°C) at Re_{50} (mean ± SE; mm) in *Clarias gariepinus*. Data fitted by the equation $y = ax + b$. Rearing with exogenous feeding (solid line): ◊, 1st spawning; ♦, 2nd spawning. Rearing without exogenous feeding (dashed line): ×, 3rd spawning; ○, 4th spawning; ●, 6th spawning, R^2, correlation coefficient

The total cumulative survival rate of individuals during the course of the study period (Fe–Re_{50}) significantly differed among water temperatures [$F_{(5,18)} = 33.6$, $P < 10^{-4}$], and ranged from 0-45%. The highest survival rate was achieved at 22.9–30.3°C. All larvae died at temperatures of 17.5, 17.4, 18.9, 33.2, 35.2 and 35.6°C (Fig. 19).

Discussion

The present study investigated the effect of water temperature on the early ontogenetic development of *C. gariepinus*. In accordance with results of Adamek (1994), Kamler et al. (1994), Haylor and Mollah (1995), and Hamáčková et al. (2007) the association of early ontogenetic development with water temperature was found.

Length of the incubation period (Fe-H_{50}) was inversely proportional to the water temperature, as was previously reported for *Cyprinus carpio* (Linnaeus, 1758) (Peňáz et al., 1983), *Coregonus albula* (Linnaeus, 1758) (Luczyński and Kirklewska, 1984), *Salmo trutta* (Linnaeus, 1758) (Raciborski, 1987).

Based on the interspecific comparisons of temperature of biological zero (t_0) and number of effective degrees days (D°_{eff}), *C. gariepinus* is a thermophilic fish species with a short incubation period and a high temperature at which ontogeny is theoretically arrested.

In accordance with Kamler et al. (1994) and Haylor and Mollah (1995), the length of the hatching period (H_5–H_{95}) was inversely proportional to the water temperature. The synchronization of hatching thus increased with rising water temperature.

Length of both endogenous nutrition (H_{50}-S_{50}) and yolk sac resorption (H_{50}-Re_{50}) periods declined with rising water temperature, as was previously reported by Viveen et al. (1986), Haylor and Mollah (1995). And prolongation of the yolk sac resorption period as much as six-fold was recorded in larvae without exogenous feeding, in comparison with larvae fed with exogenous food.

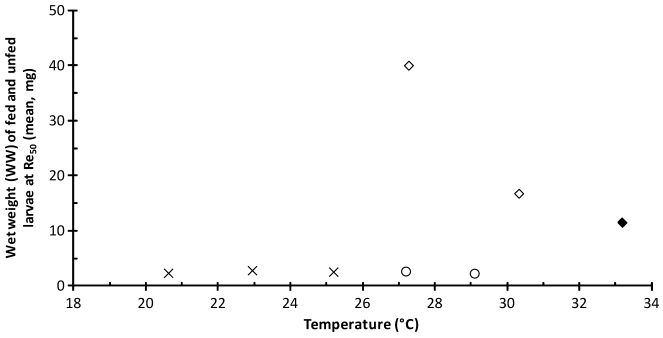

Fig. 16. Individual wet weight (WW) of fed (1st and 2nd spawnings) and unfed (3rd and 4th spawnings) larvae with water temperature (°C) at Re_{50} (mean; mg) in *Clarias gariepinus*. Rearing with exogenous feeding; ◊, 1st spawning; ♦, 2nd spawning. Rearing without exogenous feeding: ×, 3rd spawning; ○, 4th spawning; R^2, correlation coefficient

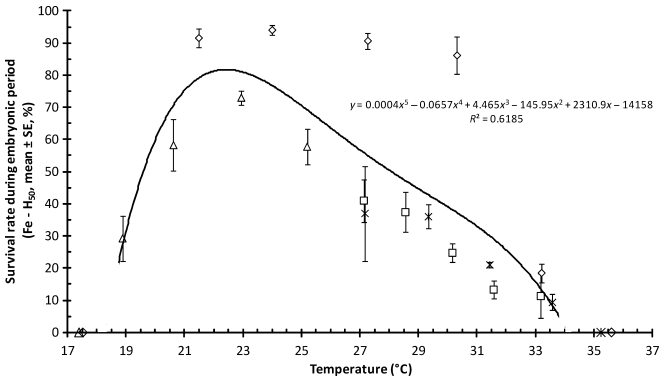

Fig. 17. Survival rate during embryonic period ($Fe–H_{50}$; mean ± SE; %) with water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = ax^5 + bx^4 + cx^3 + dx^2 + ex + f$. ◊, 1st spawning; □, 2nd spawning; △, 3rd spawning; ✳, 5th spawning; R^2, correlation coefficient

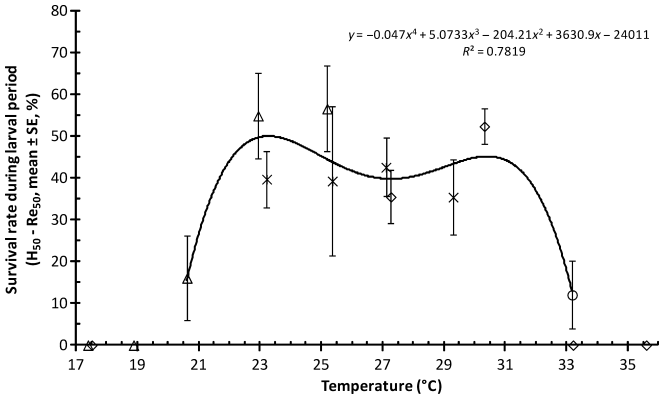

Fig. 18. Survival rate during larval period ($H_{50}–Re_{50}$; mean ± SE; %) with water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = ax^4 + bx^3 + cx^2 + dx + e$. ◊, 1st spawning; □, 2nd spawning; △, 3rd spawning; ◊, 6th spawning; R^2, correlation coefficient

In accordance with Kamler et al. (1994), the size of hatched *C. gariepinus* larvae reached a higher ontogenetic stage (larger size) at lower water temperatures in contrast to the individuals reared at higher water temperatures.

In the present work, the size of *C. gariepinus* larvae decreased with rising water temperatures at S_{50}. At Re_{50}, the size of larvae significantly differed depending on whether larvae were provided an exogenous food supply. The fed larvae

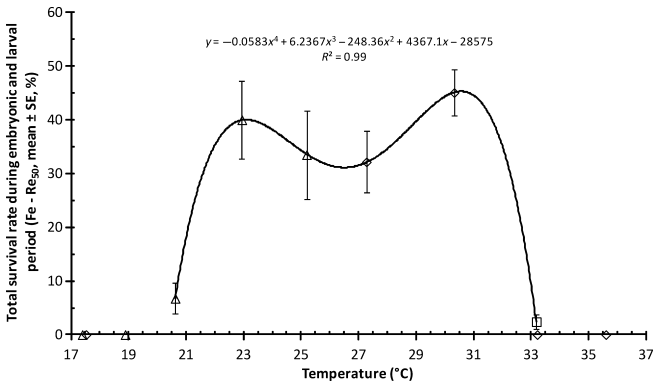

Fig. 19. Total survival rate during embryonic and larval period (Fe–Re_{50}; mean ± SE; %) with water temperature (°C) in *Clarias gariepinus*. Data fitted by the equation $y = ax^4 + bx^3 + cx^2 + dx + e$. ◊, 1st spawning; □, 2nd spawning; △, 3rd spawning; R^2, correlation coefficient

at Re_{50} reached, on average, twice the size of the unfed larvae.

At hatching, yolk sac volume of *C. gariepinus* larvae was positively correlated with size of spawned eggs. This is in accordance with the report of Peňáz (2001) that egg size and yolk sac volume influences, not only the size of hatched individuals, but also the morphological developmental stage and the time of the onset of mixogenous and exogenous nutrition.

The zone of thermal tolerance for early life history of *C. gariepinus* in terms of survival ranged from 18.9 to 33.7°C. Outside of this range, temperatures were lethal. The thermal range of 22.9–30.3°C can be considered optimal for embryonic and larval stages of *C. gariepinus*. At this range, survival ranged from 24 to 94% during the embryonic period and 35–57% during the larval period. Hamáčková et al. (2007) stated that relatively low fertilization rate (40–50% on average) varying spawning to spawning is typical for *C. gariepinus*. Survival might be most probably affected by the quality of broodstock and, consequently, by the quality of the sex products.

Other potential factors that could affect the target parameters observed in the present study were not taken into account. Mechanical and thermal shock at the start of the experiment, due to transfer of the eggs after fertilization to all the different temperatures, could be the potential factors influencing the target parameters in the present study. However, no articles on mechanical or thermal shock in *C. gariepinus* were found in the literature. Another potential factor could be the effect of light when eggs were checked every half hour during the hatching period. According to Kamler (2002), the light accelerates or conversely reduces the ontogenetic rate of fish species. However, the effect of light was not investigated in the present study.

Conclusions

Water temperature affects the early development, growth and survival of *C. gariepinus*. In terms of survival, the zone of thermal tolerance for the early life history of *C. gariepinus* ranges from 18.9 to 33.7°C, with an optimal range of 22.9 to 30.3°C.

Acknowledgements

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CHAPTER 3

EFFECT OF LIGHT INTENSITY ON EARLY ONTOGENY OF AFRICAN SHARPTOOTH CATFISH, *CLARIAS GARIEPINUS* (BURCHELL)

Prokešová, M., Stejskal, V., Matoušek, J., Kouřil, J., Baras, E. Effect of light intensity on early ontogeny of African sharptooth catfish, *Clarias gariepinus* (Burchell). Aquaculture Research (accepted for publishing 17th May, 2016).

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Effect of light intensity on early ontogeny of African sharptooth catfish, *Clarias gariepinus* (Burchell)

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Abstract

Light intensity during the early life stages of fish can have profound effects on their survival, developmental rate, yolk utilization efficiency and body size. Here, these aspects were analysed during two separate experiments (with or without exogenous food) on two distinct progenies of African sharptooth catfish, at five different light intensities (<0.1, 70, 500, 2500 and 8000 Lx; 24L:0D, 27.2°C). The duration of the egg incubation period (from 1.01 to 1.25 days post fertilization, dPF) was inversely proportional to light intensity, as hatching took place at more precocious developmental stages with increasing light intensity, i.e. at significantly ($P < 0.05$) shorter body length and slightly more abundant remaining yolk at 8000 Lx in comparison to <0.1 Lx. At the start of exogenous feeding (4 dPF), most of these differences had vanished. During the period of mixed feeding (until the end of yolk absorption, 11 dPF), growth decreased significantly with increasing light intensity. Daily mortality rates after hatching varied very little between light intensities. Mortality during egg incubation increased significantly ($P < 0.05$) with increasing light intensity, whereas it varied very little between light intensities thereafter, with the best survival rates since fertilization until the end of yolk absorption obtained at intermediate light intensities (70–2500 Lx). These results could be useful for improving the performance of African sharptooth catfish hatcheries.

Keywords: Clariidae, yolk absorption, growth, survival, hatchery management

Introduction

The African sharptooth catfish *Clarias gariepinus* (Clariidae, Siluriformes) has become a popular and important fish for freshwater aquaculture, especially in the tropics, because of fast growth, excellent food conversion, high resistance to diseases and capacity to breathe atmospheric air, which makes this species an ideal candidate for rearing at high stocking density and waters with low oxygen levels (Appelbaum & McGeer 1998; Appelbaum & Kamler 2000; Adamek, Kamler & Epler 2011). Among the possible restrictions behind the rearing of this species is the higher mortality that can take place during its early life stages, because of low fertilization rate (Hamackova, Kouril, Masar & Turansky 2007), cannibalism among its larvae and juveniles (Smith & Reay 1991; Hecht & Pienaar 1993) and difficulties of first-feeding larvae to feed on formulated feed (Verreth & Van Tongeren 1989; Verreth 1994).

The early stages of fish are most frequently less tolerant than adults or juveniles to low water quality, especially as regards pH, oxygen and temperature (Kamler 1992, 2002; Rijnsdorp, Peck, Engelhard, Möllmann & Pinnegar 2009). In comparison to these environmental factors, the roles of light have often been overlooked, although its effects can be quite directive and manifold, as they can refer to photoperiod, colour spectrum and light intensity (Boeuf & Le Bail 1999). Light can virtually

affect all aspects of fish biology, including stress responses (Billard, Bry & Gillet 1981), activity rhythms (Schwassmann 1971; Boujard & Leatherland 1992) metabolism (Boeuf & Le Bail 1999), feeding behaviour (Tandler & Helps 1985) and gonad development (Baggerman 1980). Light may affect development of early fish stages (Blanco-Vives, Villamizar, Ramos, Bayarri, Chereguini & Sánchez-Vázquez 2010; Villamizar, Blanco-Vives, Migaud, Davie, Carboni & Sánchez-Vázquez 2011). It can influence their incubation period (Downing & Litvak 2002) hatching rate (Helvik & Walther 1992; Ellis, Watanabe, Ellis, Ginoza & Moriwake 1997), yolk utilization efficiency (Watanabe, Feeley, Ellis & Ellis 1998), size of larvae (Ellis *et al.* 1997; Watanabe *et al.* 1998; Downing & Litvak 2002), first feeding (Downing & Litvak 2001; Carton 2005), survival rate (Ellis *et al.* 1997) and occurrence of deformities (Bolla & Holmefjord 1988).

The sharptooth catfish is primarily a nocturnal feeder, which relies little on vision (Hecht & Appelbaum 1987). Its larvae and juveniles often exhibit lower performances under permanent or bright light than under dim light or permanent darkness, because of enhanced aggression or avoidance of brightly lit areas, resulting in lower access to food, slower growth, higher growth heterogeneity and more frequent cannibalism (Britz & Pienaar 1992; Appelbaum & McGeer 1998; Appelbaum & Kamler 2000; Almazan-Rueda, Schrama & Verreth 2004; Mino, Metillo & Tobias 2008). Most of the knowledge on the influence of light on sharptooth catfish refers to the effect of photoperiod, then continuous light or darkness on its larvae and juveniles. However, the effects of light intensity on egg incubation and yolk utilization in sharptooth catfish have been sporadically investigated, although their knowledge can contribute significantly to improving the production of its early stages.

Therefore, the present study was aimed to provide information about the effect of light intensity on the egg incubation period, hatching process, endogenous feeding period, first ingestion of food particles, yolk absorption period, size and mortality rate during early development of sharptooth catfish.

Materials and methods

Biological material

The fish used in the present study were offspring of sharptooth catfish broodstock, originally imported from Hungary (HAKI Szarvas) in 2015, and raised at 27°C in water recirculating systems in the experimental facilities of Institute of Aquaculture and Protection of Waters. The males and females used here averaged 1.3 ± 0.1 and 1.2 ± 0.2 kg respectively. Technique of the hormonally induced artificial reproduction (Kouril, Drozd, Prokesova & Stejskal 2013) was followed. Fish gametes were obtained by, using Ovopel pellets (containing GnRH analogues and dopaminergic inhibitor metaclopromide; Agrofish, Hungary, one pellet per kg of body weight) that were injected into the muscles of females about 12 h before ova collection. No hormonal treatment was used for males. When ovulated eggs were ready to be collected from the females (as verified by gentle hand stripping), males were killed with a lethal dose of anaesthetics (clove oil, 4 mL L^{-1}), their testes and seminal vesicles were collected and cut into small pieces. Sperm was separated from tissues with a sieve, then poured over and mixed gently with ovulated eggs, using the dry fertilization technique. Thereafter, gametes were activated by adding water from the hatchery.

Design and experimental conditions

Two experiments (E1 and E2) were conducted sequentially, on distinct progenies from different pairs of broodfish. Both experiments had similar designs and took place in the same indoor water recirculating system under stable environmental conditions (27.2 ± 0.7°C, pH 7.88 ± 0.24, 7.66 ± 0.2 mg L^{-1}) that can be considered as optimal for the incubation of sharptooth catfish eggs (after Kamler, Szlaminska, Kuczynski, Hamackova, Kouril & Dabrowski 1994). Water temperature was recorded every hour with thermal data loggers (RT-F53; Qi Analytical Ltd., Prague, Czech Republic, accuracy 0.1°C). Oxygen level and pH were daily measured by multi-metre HQ40 (Hach Ltd., Prague, Czech Republic).

In both experiments, day length was 24L:0D. Five light intensities were evaluated, each of them in a separate chamber isolated by opaque plastic curtains and containing a single rearing tank ($720 \times 1000 \times 120$ mm, 70 L). Desired light intensities (<0.1, 70, 500, 2500 and 8000 Lx) were obtained using EasyLED lamp with LED lighting system placed 300 mm above water surface and tuned with a rheostat (Aquatlantis Inc., Lordelo, Portugal, 36 W, 6800 K). LED were

preferred to alternative light sources (e.g. incandescent lamps) to avoid light-dependent heating of the water. Targeted light intensities were actually obtained (as verified with a Luxmeter, CEM, Shenzhen Everbest Machinery Industry Co. Ltd., Shenzhen, China), but only in the centre of each experimental chamber, just under the LED lamp. Further away, light intensity was lower, but nevertheless without any overlap between experimental conditions (corresponding ranges of <0.1, 35–70, 200–500, 1000–2500, 3500–8000 Lx). For the sake of consistency, all experimental containers were always placed as close as possible to the centre of the experimental chamber, where light was brightest.

In both experiments, freshly fertilized eggs were transferred in each of the five rearing tanks at different light intensities, in two types of incubation structures: A fully immersed net (450 × 450 mm, mesh size of 0.5 mm), where about 10000 eggs (15 g) were poured, and three (E1) or four (E2) partly immersed cylindrical glass containers (Ø150 × 70 mm, 300 mL), each of them filled with stagnant water gently aerated with an air stone and containing a known amount (30–50) of eggs. Egg number varied slightly between containers, because the adhesive nature of sharptooth catfish eggs complicates sampling and inappropriate separation of eggs attached to each other could result in damage the chorionic membrane or embryos. These containers were examined every 30 min after the age of 15 h post fertilization (hereafter hPF), and served to measure the survival rate from fertilization to hatching. Because of rather high mortality during the first stages of egg incubation (see Results), the study of hatching dynamics during E2 was pinpointed as follows. About 5 h before the presumed hatching time at a particular light intensity (as inferred from preliminary observations during E1), three replicate batches of 20 live eggs each were counted and transferred into Petri dishes (Ø50 × 10 mm, 15 mL) and examined every 30 min until the last hatching event.

When the hatching process was completed at a particular light intensity, the net was removed and all hatchlings were collected. Three groups of 50 fish each (E1 or 30 fish, E2) were placed into three cylindrical containers (Ø150 × 70 mm, 300 mL) for daily measurements of survival until the end of the experiment. In parallel, two large groups of fish (about 300 individuals each) were placed into larger containers (Ø250 × 100 mm, 700 mL) for collecting samples at defined developmental stages. Larger amounts of fish were needed here, as sampled fish were not returned to their rearing infrastructures after measurement. Fish were selected at random, except for individuals with obvious morphological deformities (e.g. deformities of skeletal tissues, pericardial oedema – Teh, Deng, Teh & Hung 2002), which were discarded. All five containers were filled with stagnant water, which was gently aerated with an air stone, and renewed every day.

During E2, no food was given, and the experiment was over after all fish had fully absorbed their yolk (6 days after hatching). During E1, larvae were fed decapsulated cysts of *Artemia salina* (L.) nauplii, which were distributed by hand in slight excess, five times a day (from 8 a.m. to 6 p.m.), starting at the age of 3 dPF. Experiment E1 was stopped at 11 dPF.

Measurements and calculations

In each experiment, and at every light intensity, eggs were sampled just after fertilization and fish were sampled every day from hatching until the end of the experiment. In every case, about 10 fish were sampled, anaesthetized with eugenol (0.01–0.05 mL L^{-1}, depending on fish age) and photographed in profile view under the dissection microscope (SZX16; Olympus, Tokyo, Japan). Digital photographs were analysed using the software QUICK PHOTO MICRO 3.0 (Promicra, Ltd., Prague, Czech Republic). Total body length (TL) was the preferred measurement for fish size, as it could be measured consistently at all life stages, in contrast with standard body length, which could not be measured before the flexure of the notochord. The yolk sac volume (YSV) of eggs and larvae was estimated from its length (L) and depth (D), which were measured manually to the nearest pixel, using the following equation (Blaxter & Hempel 1963): $V = (\pi/6)\cdot L\cdot D^2$. Total body length (TL) was measured in hatchlings. The mean wet body weight (WW) of fish was obtained from the weighing (nearest 0.1 mg) of batches of 10 individuals.

The duration of the egg incubation period (τ) was defined as the time elapsed between egg fertilization until 50% of the individuals had hatched. The developmental rate (V) during the egg incubation period was calculated as $V = \tau^{-1}$ (Kamler 2002). Survival rates were calculated separately

for the following intervals: from fertilization (F) to hatching (H), from hatching to start of exogenous feeding (S), and from the start of exogenous feeding until the end of yolk absorption (RE).

Results

Fertilized eggs in experiment E1 were significantly larger than during experiment E2 (WW = 1.73 ± 0.02 versus 1.64 ± 0.02 mg; unpaired *t*-test, $t = 2.87$, d.f. = 7, $P = 0.02418$). In view of this difference, results from the two experiments were analysed separately, as egg weight and diameter are known to influence significantly the durations of egg incubation and yolk absorption periods (Pauly & Pullin 1988; Teletchea, Gardeur, Kamler & Fontaine 2009).

Hatching (H)

For logistic reasons, it was impossible tracking accurately the hatching dynamics at all five light intensities, so it was decided to focus on the lowest, highest and intermediate light intensities. The duration of the egg incubation period decreased significantly with increasing light intensity (one-way ANOVA, $F = 10.62$, d.f. = 8, $P = 0.0107$), from 29.92 ± 1.13 h (mean ± S.D.) at <0.1 Lx to 24.25 ± 1.42 h at 8000 Lx (Fig. 1). The corresponding embryonic rates ranged from 0.803 ± 0.030 to 0.992 ± 0.057 $days^{-1}$ respectively. Yet the H_{50} varied between light intensities, while the hatching dynamics did not differ substantially, as in all situations, 90% of the eggs (i.e. from H_5 to H_{95}) hatched within about 12 h (Fig. 1).

In both experiments, fish size at hatching decreased with increasing light intensity (Fig. 2a and b; E1: $F = 17.83$, d.f. = 97, $P = 0.0001$; E2: $F = 38.38$, d.f. = 121, $P = 0.0001$). Yolk sac volume (YSV) also varied significantly with ambient light intensity (E1: $F = 3.20$, d.f. = 97, $P = 0.0164$; E2: $F = 15.29$, d.f. = 121, $P = 0.0001$), but in different ways during the two experiments (Fig. 3a and b). In contrast with the situation for fish body length, there was no consistent decrease or increase in YSV with increasing light intensity. The wet body weight of hatchlings did not differ significantly between light intensities (E2: $F = 1.42$, d.f. = 14, $P = 0.2953$), and averaged 1.29 ± 0.07 mg during E2.

Start of exogenous feeding

At all light intensities, exogenous feeding during E1 started on the fourth day after fertilization. By then, fish TL still varied significantly with light intensity ($F = 5.61$, d.f. = 99, $P = 0.0043$), but differences were proportionally more tenuous than at hatching, and concerned exclusively fish raised under 8000 Lx, which were slightly smaller than others i.e. 7.39 ± 0.25 versus 7.71 ± 0.17 mm; Fig. 4a). Likewise, the remaining yolk at the start of exogenous feeding was significantly higher at 8000 Lx than at lower light intensities (YSV of 0.10 versus <0.65 mm^3; $F = 3.84$, d.f. = 96, $P = 0.0062$; Fig. 4b). By contrast, fish body weight did not vary significantly between light

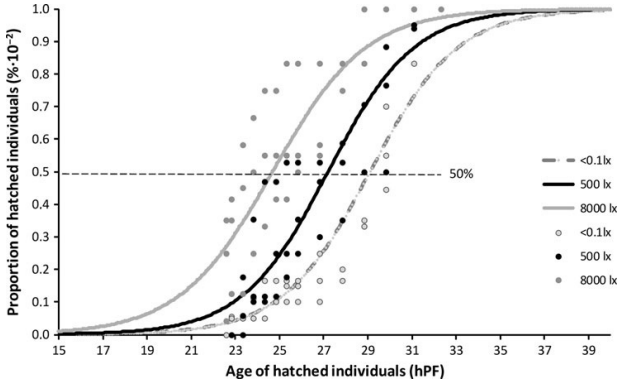

Figure 1 Hatched individuals ($\%\cdot10^{-2}$) plotted against hatching time (hPF) in dependence on light intensity (Lx) in *C. gariepinus*.

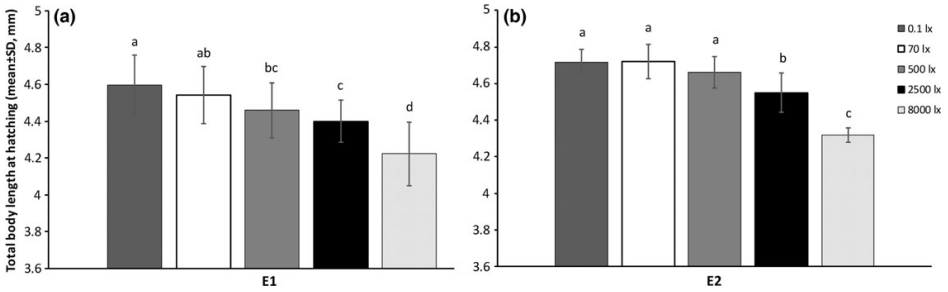

Figure 2 (a, b) Total body length of *C. gariepinus* at hatching (mean ± SD, mm) in dependence on light intensity (Lx) during experiments E1 and E2. Groups with various superscripts (a, b, c, d) differ significantly (Tukey HSD test, $P < 0.05$) from each other.

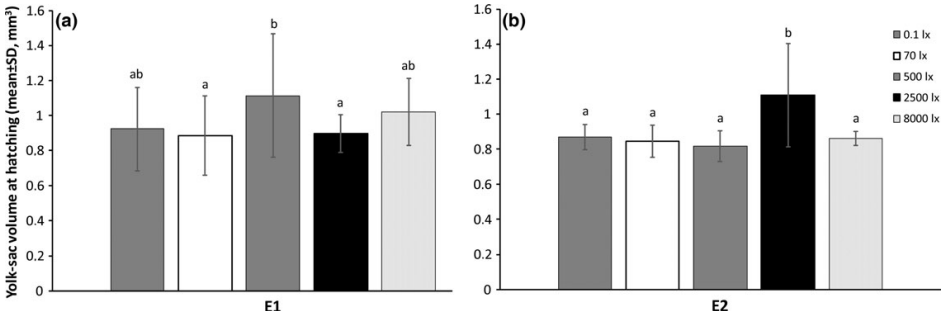

Figure 3 (a, b) Yolk-sac volume of *C. gariepinus* at hatching (mean ± SD, mm^3) in dependence on light intensity (Lx) during experiments E1 and E2. Groups with various superscripts (a, b) differ significantly (Tukey HSD test, $P < 0.05$) from each other.

intensities ($F = 0.21$, d.f. = 9, $P = 0.9219$) and averaged 3.85 ± 0.08 mg.

End of yolk absorption

At the time of complete yolk absorption (11 days after fertilization), the body length and body weight of fish having fed exogenously (E1) were both inversely proportional to light intensity (TL: $F = 19.54$, d.f. = 95, $P = 0.0001$; WW: $F = 6.76$, d.f. = 7, $P = 0.0481$; Fig. 5a and b). It should be noted that growth in this particular experiment was very slow, as the fastest growing fish (at 0.1 Lx) did not even double their WW after eight feeding days.

Regarding the fish receiving no exogenous food (E2), there were no effect of light intensity on the total body length or body weight at the end of yolk absorption (7 after fertilization; TL: $F = 1.18$, d.f. = 149, $P = 0.3223$; WW: $F = 2.72$, d.f. = 14, $P = 0.0910$). These body lengths are most similar to those of fish from E1 at the start of exogenous feeding, while the body weight was about 15–20% lower.

Mortality rates

In present study, mortality from the egg incubation period was much higher than during other ontogenetic intervals ($F = 160.77$, d.f. = 14, $P = 0.0001$), in spite of the brief duration of this period (about 1 day) in comparison to others (Fig. 6). Daily mortality during egg incubation significantly increased with increasing light intensity (E1: $F = 9.20$, d.f. = 19, $P = 0.0006$; E2: $F = 6.94$, d.f. = 19, $P = 0.0023$). It averaged 60.80 ± 8.91% and 67.20 ± 9.03% in E1 and E2 respectively.

Mortality from hatching to the start of exogenous feeding was lower than before and varied significantly between light intensities (E1: $F = 3.52$, d.f. = 14, $P = 0.0484$), being lowest (3.73 ± 0.01%) at 500 Lx and highest (11.73 ± 1.77%) at 8000 Lx.

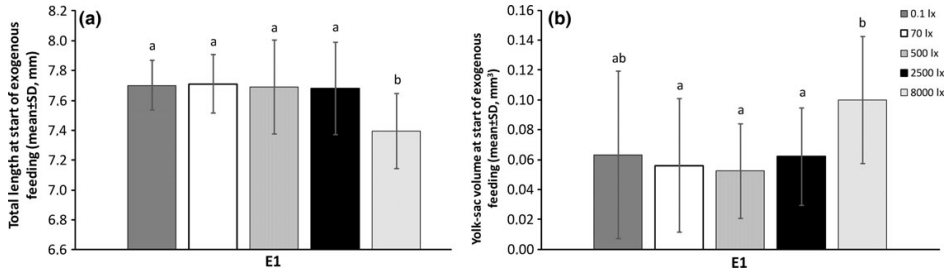

Figure 4 (a, b) Total body length (mean ± SD, mm) and yolk-sac volume (mean ± SD, mm^3) of *C. gariepinus* at start of exogenous feeding in dependence on light intensity (Lx) during experiment E1. Groups with various superscripts (a, b) differ significantly (Tukey HSD test, $P < 0.05$) from each other.

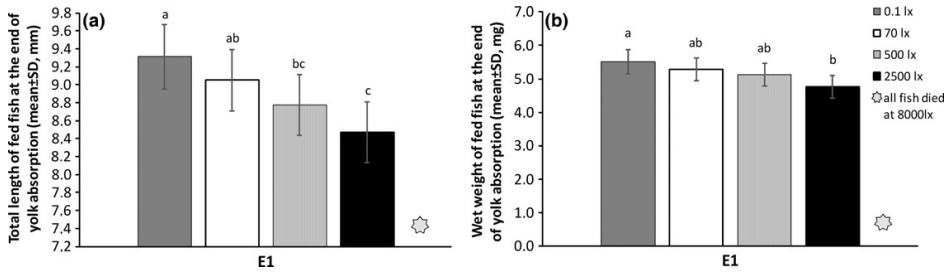

Figure 5 (a, b) Total body length (mean ± SD, mm) and wet weight (mean ± SD, mg) of *C. gariepinus* at the end of yolk absorption in dependence on light intensity (Lx) during experiment E1. Groups with various superscripts (a, b, c) differ significantly (Tukey HSD test, $P < 0.05$) from each other.

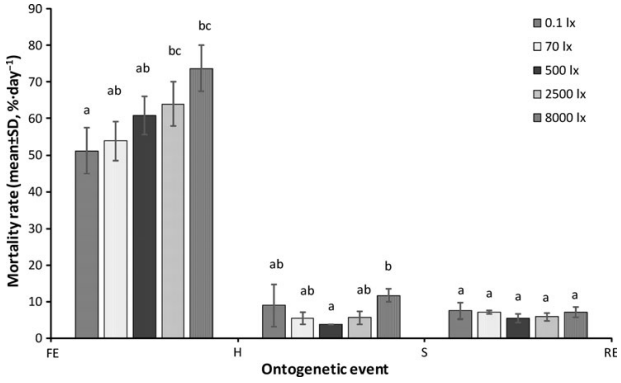

Figure 6 Mortality rate (mean ± SD, $\%\cdot day^{-1}$) of *C. gariepinus* at ontogenetic intervals FE-H, H-S and S-RE in dependence on light intensity (Lx) during experiment E1. Groups with various superscripts (a, b, c) differ significantly (Tukey HSD test, $P < 0.05$) from each other within a single ontogenetic interval.

During the period of mixed feeding (i.e. until the end of yolk absorption), the mortality rate in E1 did not differ significantly between light intensities (E1: $F = 1.11$, d.f. = 14, $P = 0.4041$) and averaged 6.60 ± 0.87% in E1.

Discussion

The present study provided evidence that light intensity can affect the survival and development of embryos and yolk sac larvae of sharptooth catfish, in a similar way as after the start of exogenous feeding (Appelbaum & McGeer 1998; Appelbaum & Kamler 2000), with lower performances under bright light than under dim light or darkness. Based on this study, the hatching and development of sharptooth catfish took place continuously from egg fertilization until the end of yolk absorption at all light intensities. Therefore, it can be concluded that there is no inhibitory effect

of light on its ontogeny, in contrast with other fish species (e.g. the halibut *Hippoglossus hippoglossus*; Helvik & Walther 1992) whose hatching is inhibited by lighting, whereas under darkness the inhibition of hatching is reversed.

The incubation of sharptooth catfish ranged from about 24 to 30 h at 27°C in the present study. This range compares well with the values 22–33 h observed by many authors for this species at similar temperatures 25–28°C (Legendre & Teugels 1991; Adamek 1994; Haylor & Mollah 1995). Yet, much shorter incubation periods have been observed for sharptooth catfish, but almost systematically when studying eggs with smaller diameters (e.g. 17 h at temperature of 28.5 ± 0.5°C in 1 mm eggs in Olaniyi & Omitogun 2013, versus eggs of about 1.5 mm in diameter here). It is difficult to draw any further meaningful comparison with these studies and ours regarding the effect of light intensity, as it was not measured or mentioned by these authors.

Here, the relationship between the duration of the incubation period and light intensity was found curvilinear, with eggs incubated in darkness taking about 25% longer (6 h) to hatch in comparison to those under the brightest light intensity. It is quite frequent that egg hatching under bright light takes place earlier in fish (*Paralichthys dentatus*, Watanabe *et al.* 1998; *Hippoglossus hippoglossus*, Downing & Litvak 2002), partly because lighting can increase the frequency of muscular contractions of the embryo, with more frequent movements resulting in a more precocious rupture of the chorionic membrane. Alternatively, it can be hypothesized that earlier hatching under bright light originates from higher metabolism and use of yolk reserves. The former hypothesis, i.e. that sharptooth catfish hatched at a precocious developmental stage under bright light, is largely supported by the negative correlation between light intensity and the body length of catfish hatchlings, and to a lower extent by the finding that the remaining yolk sac of hatchlings under bright light was larger than under dim light or darkness (Fig. 2a and b). At the start of exogenous feeding and at the end of yolk absorption in unfed fish, most of these differences had vanished. This further suggests that variations between hatching times at different light intensities did not chiefly originate from contrasting metabolic rates and that yolk utilization efficiency was little affected by light intensity. The sole exception to this general trend concerned fish reared under 8000 Lx, which were eventually slightly smaller than others.

The growth of sharptooth catfish larvae feeding exogenously was negatively correlated with light intensity in the present study (Fig. 4a). Similar trends were observed in several other studies on the same species (Britz & Pienaar 1992; Appelbaum & McGeer 1998; Appelbaum & Kamler 2000), and interpreted as the result of both higher propensity to forage and lower aggressiveness under low light levels in comparison to bright light. As pointed out earlier, the growth of larvae here was very slow in comparison to other studies, probably because the decapsulation process of *Artemia* cysts was improper, as many intact cysts were observed exiting the intestine of photographed larvae, resulting in very poor food absorption. Nevertheless, the results indicate that the light-dependent growth patterns observed in situations of rapid growth also apply to situations of slow growth. This indirectly suggests that differences in aggressiveness might suffice to produce such patterns, as the motivation to feed was supposedly high under all light intensities in contexts of very slow growth and low food absorption rates.

Proportionally, the mortality of sharptooth catfish during the egg incubation period (>50% day^{-1}) was much higher than in yolk sac larvae feeding endogenously (7.5% day^{-1}) and after the start of exogenous feeding (6.7% day^{-1}). It is possible that the high mortality during the embryonic period here partly reflected a rather low fertilization rate, as suggested by the observation of numerous decaying eggs no later than 8 h after fertilization. During egg incubation, the mortality of sharptooth catfish was proportional to light intensity, for reasons that remain to be elucidated. Studies on the eggs of other fish species have produced contrasting trends, sometimes with mortality being negatively correlated with light intensity (e.g. *Rhombosolea tapirina*, Hart, Hutchinson & Purser 1996), whereas in other instances, egg mortality was higher under low light while the proportion of deformed hatchlings increased with ambient light intensity (e.g. *Hippoglossus hippoglossus*, Bolla & Holmefjord 1988; *Epinephelus striatus*, Ellis *et al.* 1997). After hatching, the mortality of sharptooth catfish in the present study was independent from light intensity, nevertheless with the best survival rates observed under intermediate light intensities, and the worst scores under the brightest light or darkness. All in all, the

combination of mortality rates from fertilization until the end of yolk absorption clearly indicates that rearing under 8000 Lx produces overall survival rates almost twice as low than under 500 or 2500 Lx. Rearing under dim light (70 Lx) or darkness (<0.1 Lx) produced slightly lower survival rates than under these intermediate light levels.

To sum up, the present study provided evidence that rearing under bright light (8000 Lx) is truly detrimental to sharptooth catfish during all life stages until the end of yolk absorption, as it produces much lower survival, and slightly smaller fish than under lower light levels. If egg incubation and rearing of yolk sac larvae take place in different infrastructures, then it is recommended incubating eggs in darkness, then rearing larvae under dim light or intermediate light levels, as these will provide the best survival rates. If a single light intensity must be chosen for all life stages until yolk absorption, then it is recommended using light intensities of 70–500 Lx rather than darkness or bright light, as they can facilitate the work of hatchery operators while minimizing electricity costs.

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CHAPTER 4

INTENSIVE REARING OF AFRICAN SHARPTOOTH CATFISH (*CLARIAS GARIEPINUS*)

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Jihočeská univerzita
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in České Budějovice

Intensive rearing of African sharptooth catfish (*Clarias gariepinus*)

J. Kouřil, B. Drozd, M. Prokešová, V. Stejskal

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Breeding and Environmental Aspects of Aquaculture and Hydrocenoses (GA JU 047/2010/Z)

Environmentally and hormonally induced reproduction, anesthesia, early ontogenetic development and rearing of selected endangered and economically important fish species (ME10126)

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CONTENT

1. INTRODUCTION

1.1. Taxonomy and biological characteristic

African sharptooth catfish, *Clarias gariepinus* (Burchell 1822; Fig. 1) belongs to the class Actinopterygii (ray-finned fish), order Siluriformes (catfish), Clariidae family (labyrinth catfishes) which includes about 100 species in 13 genera (Hanel & Novák, 2004). The Czech name for this species is not fixed. In Czech literature, several synonyms can be found – e.g. sumeček africký (Adámek, 1994; Hamáčková *et al*., 2007), keříčkovec červenolemý (Hanel, 1997), klarias africký (Kůrka *et al*., 2000), sumčík africký (Pokorný *et al*., 2004; Hamáčková *et al*., 2007). The present official Czech name is keříčkovec jihoafrický (Hanel & Novák, 2004).

Species of the Clariidae family inhabit stagnant freshwaters of Syria, Southeast Asia (Philippines and Java), Malaysia, Africa and Madagascar. Outside the African continent, the African sharptooth catfish is found in Asian countries of the Mediterranean coast. The northern boundary of its distribution is southern Turkey (Viveen *et al*., 1986). It was introduced and occurs in the wild as well as in Florida in the USA (de Graaf & Janssen, 1996).

Fig. 1. *African sharptooth catfish, Clarias gariepinus (Burchell 1822). Photo by J. Kouřil*

African sharptooth catfish populations occurring in various parts of Africa were originally known by different names: *Clarias mossambicus* (eastern part), *Clarias lazera* (northern and central part), *Clarias senegalensis* (western part) and *Clarias gariepinus* (southern part). However, it is always one and the same species (Teugels, 1984).

In natural habitats, the African sharptooth catfish is a species well adapted to diverse environmental conditions. It occurs in various types of African inland waters, both

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standing and slowly flowing, with an average temperature of 25 °C. It lives in both shallow and muddy waters and in deep lakes with relatively clean water. During the rainy season, it migrates to spawn into the shallow tributaries (Hecht *et al*., 1988).

The African sharptooth catfish body is torpedo-elongated without scales. Dorsal and lateral parts are dark grey to olive in colour and the ventral part is white. There are also individuals with bright spots or bright colour throughout the body. The head is dorso-ventrally flattened with a strong bone structure of the skull. There are 4 pairs of long barbels around the mouth. The dorsal fin extends to the caudal peduncle with 68–79 soft fin rays. The first fin rays of the pectoral fins are hard and serrated on the inside (Hamáčková *et al.*, 2007).

The Clariidae family is characterized by the occurrence of a suprabranchial accessory air-breathing organ formed by arborescent extensions of mucosa of the branchial cavity above the gill arches (Baruš & Oliva, 1995). The arborescent organ allows the survival of labyrinth catfishes in waters with low or zero oxygen content (adaptation to drought, when the water remains only in the deepest places of periodically flooded areas in places of its natural distribution). The ability to breathe also atmospheric oxygen is one of the major reasons why African sharptooth catfish was successfully introduced into intensive aquaculture (Hamáčková *et al*., 2007).

Due to the construction of its body and adaptability, this species is able to consume a wide range of food from tiny zooplankton to fish prey reaching almost half of its body length. Its short and extended esophagus allows it to eat even larger prey. In the stomach, the food is diluted and continues into the intestine which is simple, thin and relatively short. Due to this fact, African sharptooth catfish is dependent on the intake of high-protein food. The digestive system develops relatively quickly; it allows early transfer to dry starter food in case of intensive reared fish, unlike some other fish species (Hecht *et al*., 1988).

Because of the great adaptability and wide range of inhabited waters, the information about growth rate of African catfish differs considerably. In nature, under optimal conditions, it reaches usually about 200–300 mm of the total length in the first year of life. In subsequent years, the yearly growth is 80–100 mm. In nature, it grows to the maximum total length of 140 cm and weight of 40–60 kg. The largest specimens are found primarily in large turbid rivers (Hecht *et al*., 1988).

The African sharptooth catfish is characterized by mostly evening and nocturnal activity of food intake. In nature, it feeds especially predatory on plankton and bentos (various invertebrates and their developmental stages) and amphibians. Juveniles and adults feed mostly smaller fishes, including dead fish (Hecht *et al*., 1988; Yalcin *et al*., 2000).

In experimental conditions, Adámek *et al*. (1989) studied the food selection of African sharptooth catfish with a weight of about 220 g, originally bred in intensive rearing. From eight food fish species offered with an individual total length

about 12–22% of the length of African sharptooth catfishes, catfishes preferred primarily Belica, *Leucaspius delineatus* (Heckel, 1843) and Rudd, *Scardinius erythrophthalmus* (Linnaeus, 1758). Conversely, negative selection was demonstrated in Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) and Stone maroko, *Pseudorasbora parva* (Temminck & Schlegel, 1846). Generally, the intensity and efficiency of predation was quite low with regard to the predation strategy in African sharptooth catfish. After multiple attacks the injured or dead prey was eaten during the night. Achieved feed conversion ratio (FCR) in African sharptooth catfishes fed by live fishes was 4.73 and specific growth rate (SGR) 0.39%.day^{-1}. This information partly refuted a hypothesis about the utility of African sharptooth catfish breeding in polyculture with Nile tilapia, where the African catfish should partly correct the number of fish and population density of fast reproducing Nile tilapia (Adámek *et al*., 1989).

Sexual dimorphism is strongly developed in African sharptooth catfish. Males are characterized by longer sexual papilla of conical shape, females have star-shaped papilla and a visibly enlarged abdominal part before the spawning period (Hamáčková *et al*., 2007) – see Fig. 2 and 3. In nature, at the beginning of the rainy season, the broodstock migrate into vegetated shallow tributaries where they spawn on plant substrate. Parental care was not recorded, generation fish return back into their original habitat. Several months after hatching, the progeny stays in vegetated, shallow waters and migrates downstream into larger streams and lakes at the beginning of dry season.

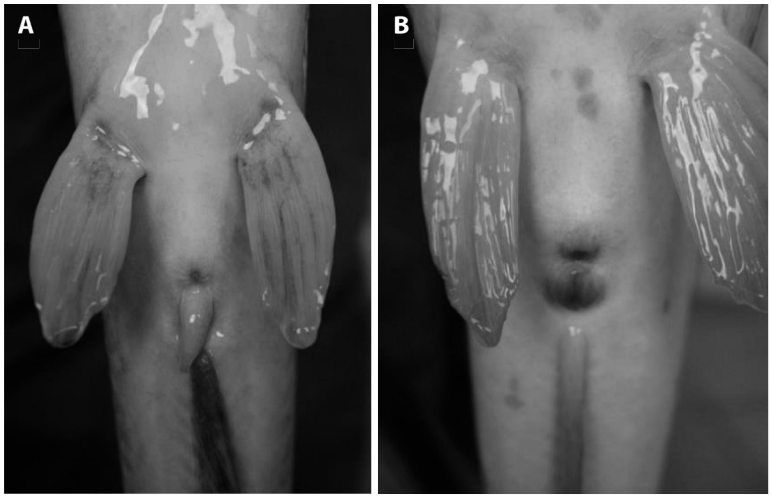

Fig. 2. *Detail view of organization of male and female genital papilla in African sharptooth catfish (A and B, respectively). Photo by J. Kouřil*

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Fig. 3. *Male (left) and female (right) of African catfish. Photo by J. Kouřil*

1.2. Rearing methods

Studies of Dutch authors (Hogendoorn, 1979, 1980, 1981; Hogendoorn & Vismans, 1980; Hogendoorn *et al.*, 1983; Viveen *et al.*, 1986) were crucial for development of African sharptooth catfish breeding. Soon, intensive rearing was spread from the Netherlands to many other countries in Europe (especially into Hungary, but also into Germany and Poland) and elsewhere in the World (Fig. 4 and 5). The main reasons for its rearing in aquaculture are high adaptability to the environment (except low temperature), low demands on oxygen, high stocking density, great growth rate and high muscle quality (high dietetic value, excellent taste and lack of muscle „Y" bones). It was introduced into the Czech Republic in 1989 (Pokorný *et al.*, 2004).

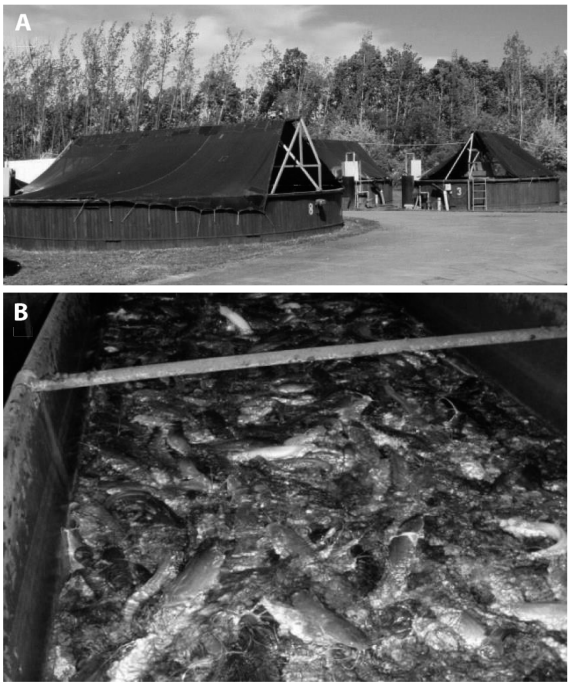

Fig. 4. *Intensive farming of African sharptooth catfish in Szarvas (HAKI – Research Institute for Fisheries, Aquaculture and Irrigation) in Hungary: view in the nursery hall outside (A), tank with farmed fishes (B). Photo by J. Kouřil*

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Fig. 5. *Exterior (A) and interior (B) of the original agricultural building which was adapted and rebuilt as a farm with intensive rearing of African sharptooth catfish (firm „Krolestwo ryb") in Pielgrzymowice in Poland. Photo by J. Kouřil*

Rearing of African sharptooth catfish is not possible in outdoor tanks with a natural water temperature during the greater part of the year, due to low water temperatures. However, either year-round breeding in water-flow or closed-water systems with water temperatures above 20 °C, or a combination of summer rearing in ponds (Adámek, 1994; Adámek & Sukop, 1995) with aforementioned breeding in systems with heated water is feasible. Thanks to the possibility of breeding African sharptooth catfish in high stocking densities, this fish belongs among to the most suitable species for

breeding in recirculation aquaculture systems (hereinafter RAS; Hamáčková *et al.*, 2007). Intensive RAS fish rearing represents an important alternative to intensive fish production in flow water systems and pond rearing (Kouřil *et al.*, 2008). As evidence, the development of African sharptooth catfish production in Hungary may be considered, where it reaches about 15% of total fish aquaculture production. In RAS optimal rearing conditions can be maintained in terms of both water quality and dosing feed. Recirculation aquaculture systems are systems with partially or completely closed water circulation. They are independent of the external environment, they have low demands on the amount of water and limited build-up area. In such devices, all water, or at least part of water used for fish farming is purified and modified in such way that it can be reused. Fish excrements and any feed remnants are removed from the water by sedimentation and mechanical filtration. The end product of protein metabolism – ammonia, is oxidized biologically (not chemically) by the use of biological nitrification filters. Ammonium is converted into nitrites and subsequently to nitrates by nitrification process. Through a denitrification process, these ions can be converted up to molecular nitrogen which escapes into the atmosphere. The next product of metabolism – carbon dioxide, has to be removed from the water by use of outgassing (with aeration or oxygenation). In this way, the dissolved oxygen in the water is also replenished. Water constantly circulates in RAS. Only, a small amount of circulating water is replaced by fresh water. Usually, it is a volume from 0.1% to 10% of the total volume of the RAS. That´s why the RAS is characterized by high fish production with the use of a very small built-up area and need for low inflow of water (Kouřil *et al.*, 2008).

The African sharptooth catfish is a very resistant fish species which is capable of inhabiting waters with very low oxygen levels thanks to its accessory air-breathing organ. According to Britz & Hecht (1987), a temperature of 26–32 °C is ideal for its intensive rearing. Outside this thermal range, temperatures reduce its growth. The African sharptooth catfish can be reared in water with higher salinity. Salinity up to 0.5% is acceptable for rearing of its fry. Regarding the survival rate, the salinity of 0.75% is suitable for this species (Britz & Hecht, 1989). According to Hamáčková *et al.* (2007), the oxygen saturation is also important for fry before the start of breathing atmospheric oxygen. In this period, oxygen saturation above 90% is appropriate. In any case it must not fall below 40%. The pH should be maintained between 6.5 and 8.0. Mortality occurs when the pH value exceeds 11 and conversely, it decreases when the pH value is below 4. According to Adámek (1994), the African sharptooth catfish survives without consequences if there is a short-term drop of temperature below 12 °C. However, the fish are affected by fungus and die during long-term decline below 15 °C. The upper lethal temperature is very high (above 40 °C). Several days after hatching (it depends on the water temperature), yolk-feeding larvae search for cover and accumulates in the dark parts of the tank. Therefore, an overshadow above the

inlet part of the rearing tank is recommended (Viveen *et al*., 1986; Hamáčková *et al*., 2007). In comparison with a permanently illuminated environment, dim conditions cause a higher survival rate of fry (Britz & Pienaar, 1992; Appelbaum & Kamler, 2000). Also, rearing of market size fish takes place either in the shade or complete darkness.

In conditions of intensive rearing, the reproduction of African sharptooth catfish is carried out by hormonally induced artificial spawning with the use of carp pituitary or synthetic hormonal preparations based on GnRH (*gonadotropine releasing hormone*). Females are highly fertile and their spawning is quite easy. The relative working fecundity (number of spawned eggs per kg of female weight) averages 100–150 thousands eggs (Adamek, 2001; Brzuska *et al*., 2004). Females sexually mature at the age of 6 to 7 months. In terms of spawning and subsequent fry rearing, the best results are achieved in females at the age of 2–3 years. Males become sexually mature at the age of 1.5–2 years. Both sexes of broodstock fish can be kept together in one tank. The optimal water temperature for rearing of broodstock is 23–25 °C (Hamáčková *et al*., 2007).

Good feed conversion of intensively reared African sharptooth catfish is dependent on the quality of food supply. The African sharptooth catfish is an omnivorous fish species characterized by a high activity of digestive enzymes (amylase, lipase and protease; Fourie, 2006). Feeding experiments performed by Hecht *et al*. (1988) demonstrated the value of a feed conversion ratio (FCR) of about 1.0 with a prerequisite for further improvement.

Although the African sharptooth catfish is classified as an omnivorous fish species, its intestine is simple, thin and relatively short, which means that it is dependent on a diet rich in protein. Based on previous feeding experiments, the best parameters of food conversion were observed by using a diet containing of 38–42% crude protein and 8–12% fat (Hecht *et al*., 1988). De Graaf & Janssen (1996) recommend a diet with 35–42% of protein and 12 $kJ.g^{-1}$ of digestible energy.

Based on later research of de Graaf & Janssen (1996), the optimal nutrient content in the dry matter of feed was defined by individual age categories of African sharptooth catfish. The optimal content of 35–40% digestible protein and 12–16 $kJ.g^{-1}$ of digestible energy is equally recommended for fry and broodstock. Market size fish should be fed by food containing of 30–35% of digestible protein and 10–14 $kJ.g^{-1}$ of digestible energy. As well, the recommended range of calcium (Ca) and phosphorus (P) content achieves 0.8–1.5% of Ca and 0.6–1.0% of P in food for fry and broodstock. In the case of market size fish, it should be 0.5–1.8% and 0.5–1.0% (Ca and P, respectively). Also, the minimum requirements for the content of some amino acids were studied. Methyonin and cystyne concentrations are about 1.2% in fry, 0.9% in market size fish and 1.0% in broodstock. Lysin content should reach 2.0 % in fry, 1.6% in market size fish and 1.8% in broodstock.

The water temperature is an important factor influencing growth and feed conversion. It significantly affects the intensity of feed intake in African sharptooth catfish. Hogendoorn *et al.* (1983) recommend feed rations in % of total biomass for individual weights (from 1 to 200 g) based on temperature (range 21–33 °C) by use of commercial feeding (protein content of 50%) for Rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792).

After the above study, the highest feed rations were recorded at temperatures in the range of 27–29 °C. Thereafter, Adamek (2001) refined these values (see Tab. 1) for rearing of African sharptooth catfish fry and juveniles (fish average weight 1–150 g). The table is supplemented with temperature, weight of reared fish and presumed relative daily weight gain. Adamek (2001) also recommended daily feed ratios for breeding of market size fish of African sharptooth catfish (Tab. 2).

Tab. 1. *Recommended relative daily feed rations (in % of the stocking biomass per day) and presumed growth rate (in % of weight gain of fish per day; numbers in brackets) in rearing of African sharptooth catfish fry and juveniles based on water temperature and individual weight of fish (Adamek, 2001).*

Water temperature (°C)	Average individual weight (g)						
	1	5	15	25	50	100	150
20	4.8 (3.5)	4.3 (3.0)	3.6 (2.5)	2.4 (1.4)	1.4 (0.7)	0.9 (0.3)	0.7 (0.3)
21	5.4 (4.3)	4.8 (3.8)	4.3 (3.2)	3.0 (2.0)	1.9 (1.1)	1.2 (0.5)	1.0 (0.4)
22	5.9 (5.2)	5.4 (4.6)	4.9 (4.0)	3.7 (2.7)	2.6 (1.5)	1.7 (0.8)	1.5 (0.7)
23	6.3 (6.1)	6.0 (5.5)	5.5 (4.9)	4.4 (3.4)	3.3 (2.1)	2.2 (1.2)	2.0 (1.1)
24	6.8 (7.1)	6.4 (6.4)	6.1 (5.8)	5.1 (4.2)	3.9 (2.7)	2.7 (1.6)	2.2 (1.4)
25	7.2 (7.9)	6.9 (7.3)	6.5 (6.6)	5.7 (5.0)	4.5 (3.3)	3.1 (2.0)	2.4 (1.8)
26	7.5(8.7)	7.2 (8.1)	6.9 (7.3)	6.1 (5.6)	5.0 (3.8)	3.4 (2.3)	2.4 (2.0)
27	7.7 (9.3)	7.4 (8.6)	7.1 (7.9)	6.4 (6.1)	5.2 (4.2)	3.5 (2.5)	2.4 (2.0)
28	7.8 (9.8)	7.6 (9 0.)	7.3 (8.2)	6.4 (6.3)	5.2 (4.3)	3.5 (2.5)	2.3 (1.9)
29	7.8(10.0)	7.6 (9.2)	7.2 (8.3)	6.3 (6.2)	5.0 (4.1)	3.2 (2.2)	2.1 (1.6)
30	7.8 (10.0)	7.4 (9.1)	7.0 (8.2)	5.9 (5.9)	4.5 (3.7)	2.8 (1.9)	1.8 (1.3)
31	7.8 (9.7)	7.2 (8.8)	6.7 (7.8)	5.4 (5.4)	3.9 (3.2)	2.3 (1.4)	1.5 (0.9)

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Tab. 2. *Recommended daily feed rations (in % of fish biomass) based on water temperature during rearing of African sharptooth catfish in market size.*

Individual weight of fish (g)	Water temperature (°C)						
	20	**22**	**24**	**26**	**28**	**30**	**32**
100–300	1.2	2	2.5	3.2	3.5	3.2	3
300–800	1	1.7	2.2	2.8	3.1	2.9	2.8

Unlike the vast majority of the other fish species, the African sharptooth catfish is characterized by muscle (meat) typically red in colour with very little fat and high protein content. From a culinary point of view, its muscle has excellent taste properties (Hamáčková *et al.*, 2007). Osibona *et al.* (2009) studied the meat composition of African sharptooth catfishes purchased from local fishermen in Lagos (Nigeria) with representation of 18.8% protein, 9.3% fat and 1.2% ash. Fourie (2006) enumerates various supply options of African sharptooth catfish for consuming purposes: whole fish (only killed), gutted fish (removed viscera and head, but fins and skin remain), processed body (without viscera, head and fins), steaks (slices 20–25 mm wide prepared by a transverse cut through processed body) and fillets (with skin or skinless).

The African sharptooth catfish is native in Africa, where it is the second most important fish species in intensive aquaculture after Nile tilapia. Demand for fish continues to increase and the production of African sharptooth catfish is an important part of the national economy of countries like Nigeria, Kenya, Cameroon, Mali and South Africa. Primarily, African sharptooth catfish is reared in small ponds in a one-year to two-year production cycle; it often is reared in polyculture with Nile tilapia. Exceptionally, African sharptooth catfish is reared in cages (Hecht *et al.*, 1988). Cage culture of African sharptooth catfish in heated waters is also carried out in Bulgaria (Kouřil, unpublished). In particular, Nigeria, Netherlands, Hungary, Kenya, Syria, Brazil, Cameroon, Mali and South Africa belong to the major global producers of African sharptooth catfish. At the beginning of 90's of the last century, several farms in Belgium, Germany and Hungary increased production of African sharptooth catfish from 5 to 200 tons per year. Some farms started to specialize in reproduction and rearing of fry, others in production of market size fish. Based on the FAO (*Food and Agriculture Organization of the United Nations*) statistics, African sharptooth catfish production in aquaculture has grown exponentially over the last thirty years. In 1980, the global production of African sharptooth catfish was about 50 tons, in 1990 already 1.5 thousand tons, in 2000 it was 5.5 thousand tons and in 2010 as much as 191 thousand tons (see Fig. 6, Pouomogne, 2012 – FAO statistics).

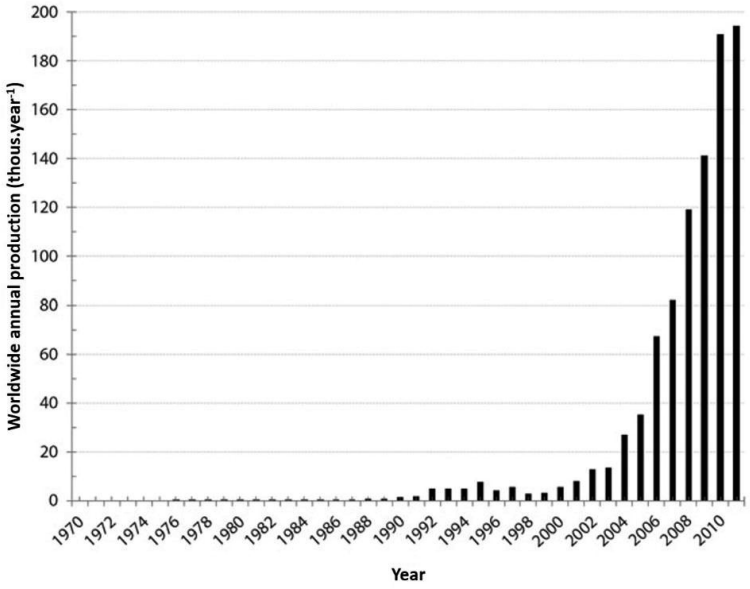

Fig. 6. *Worldwide annual production (thousand tons per year) of market size African sharptooth catfish in aquaculture in the years 1970–2011 (Pouomogne, 2012 – FAO statistics).*

Artificial spawning of African sharptooth catfish females was usually induced by hormonal stimulation by using of Common carp, *Cyprinus carpio* (Linnaeus, 1758) and exceptionally synthetically produced combined hormonal preparations were used. Carp pituitary is once injected intramuscularly or intraperitoneally at a dose of 2 to 3 mg.kg^{-1} as a suspension of crushed pituitary and dissolved in physiological saline solution. At a temperature of 25 °C, the spawning occurs after 11 h from using of carp pituitary. The water temperature significantly influences the length of latency interval (Adamek, 2001; Hamáčková *et al.*, 2007). The length of the latency period (i.e. time from injection until the egg ovulation) and length of incubation period of eggs is dependent on the water temperature (see Tab. 3).

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Tab. 3. *Length of latency interval (period from injection until spawning) and length of incubation period (time interval from egg fertilization till hatching) in African sharptooth catfish at different water temperatures (18–30 °C; Adamek, 2001).*

Temperature (°C)	Length of latency interval (h)	Length of incubation period (h)
18	21	57
21	18	46
22	15	38
23	13	33
24	12	29
25	11	27
26	10	25
27	9	23
28	8	22
29	7.5	21
30	7	20

Currently, synthetic hormonal preparations are used increasingly for induction of artificial spawning in various fish species in the Czech Republic. Utilization of GnRH – Kobarelin (D-Ala^{6}, Pro^{9} NHEt-mGnRHa) and Lecirelin (D-Tle^{6}, Pro^{9} NHEt-mGnRHa), as well as combined preparation, e.g. Ovopel (AgroFish firm, Hungary) containing the above mentioned analogue GnRH and dopaminergic inhibitor metoclopramide is proved. The Hungarian preparation Ovopel is applied once intramuscularly or intraperitoneally in doses of 10–40 µg.kg^{-1} (1 pellet per 1 kg of female). After the Ovopel injection, female ovulation occurs in 12–13 h at a temperature of 24–25 °C (Kouřil *et al*., 2011).

After the hormonal injection, it is absolutely necessary to retain the females separately (one fish per tank) in perfectly covered tanks, because of their increased aggressiveness and attempts to escape from the tank. Males can be kept together before the spawning. They should not be fed 1–2 days before the planned injection. The optimal water temperature for reproduction is 25–27 °C. Before the artificial spawning, females have to be anaesthetized using clove oil (at a dose of 0.04–0.05 ml.l^{-1} of water) or 2-phenoxyethanol (at a dose of 0.3–0.5 ml.l^{-1} of water). Before hand stripping of a female, the ventral body part and fins have to be dried. The relative weight of the total spawned eggs is 10–20% of female weight before

spawning. Spawned eggs range from yellow-green, green to brown-green colour. The weight of an egg is about 1.4 mg, i.e. 1 kg of dry mass of spawned unswollen eggs contains approximately 700 thousand eggs (Hamáčková *et al.*, 2007; Kouřil *et al.*, 2011).

Milt is obtained from just killed males by preparation of gonads. Mature gonads should be white or cream in colour. Dissected gonads have to be dried and then cut by scissors. Consequently, pieces of gonads will be sieved through a dry sieve or inert textile directly on the mass of eggs which are divided after 200–300 g separately into dry containers. Equally, milt can be firstly collected into glass containers and subsequently used. Egg fertilization is carried out in containers which were used during spawning. When eggs and sperm are mixed together, water is poured and the mixture of water and gametes is mixed again. After a further 2–5 min, fertilized eggs are washed with water and impure water with sperm remnants is rapidly decanted. Thereafter, fertilized eggs should be properly spread in an incubation tank, so that they stick on the submerged sieve.

African sharptooth catfish eggs can be also incubated in Zuger jars. In this case, eggs have to be unstuck. However, there are fish hatcheries where water composition allows egg incubation without unsticking. Clay or tannin suspension (tannin concentration 0.7–1 $g.l^{-1}$ of water) can be used for egg unsticking. Before preparation of the suspension, tannin is firstly dissolved in amount of warm water. In this solution, the unsticking of eggs is realized by two short baths, both for 20 seconds. Between these baths, as well as after completion of the unsticking process, the eggs are decanted by a sufficient volume of water. Then, the eggs are placed into incubation jars and the water flow is adjusted (Hamáčková *et al.*, 2007; Kouřil *et al.*, 2011).

Because African sharptooth catfish is a tropical fish species, there are numerous modifications of its rearing methods. In countries of natural distribution, it is usually reared in non-flow ponds or various scphisticated breeding systems when it is fed by fish, waste of various origins or fodder mixtures. Currently, it is reared in countries of temperate climate zones (i.e. less suitable climate areas) in various breeding systems. In these countries, rearing of African sharptooth catfish can take place seasonally in ponds (only in summer) and in tanks and cages in flow-water systems with heated water (thermal water, cooling water from industry). Its controlled reproduction is usually provided using hormonal stimulation with subsequent artificial spawning and egg incubation. Fry rearing is a separate section of African sharptooth catfish breeding for the first two months after hatching that is more difficult due to higher requirements for a sufficient amount of dissolved oxygen before the onset of additional breathing, rearing hygiene and adequate nutrition. Due to cannibalism, a loss of fish can occur up to an individual weight of 200–300 g. Up to its market size, African sharptooth catfish can be reared in extreme stocking densities (up to 300–400 $kg.m^{-3}$) in flow-water or recirculating systems at relatively low oxygen level and high organic load. This is different from other intensively reared fish species, for which the stocking density

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generally must not exceed 100 kg.m^{-3} during their rearing. The African sharptooth catfish grows rapidly, efficiently utilizes the food, it is characterized by high quality of product without intermuscular bones. These properties make it a very attractive fish species for intensive farming. Its production cycle is shown in Fig. 7.

By determining the consumer value of African sharptooth catfishes (an average weight of 340 g), Krupka (1998) recorded yield of fillets without skin of about 52%, the relative weight of body without head, fins and viscera reached 70% and the relative weight of body without head, fins, viscera and skin was about 62%.

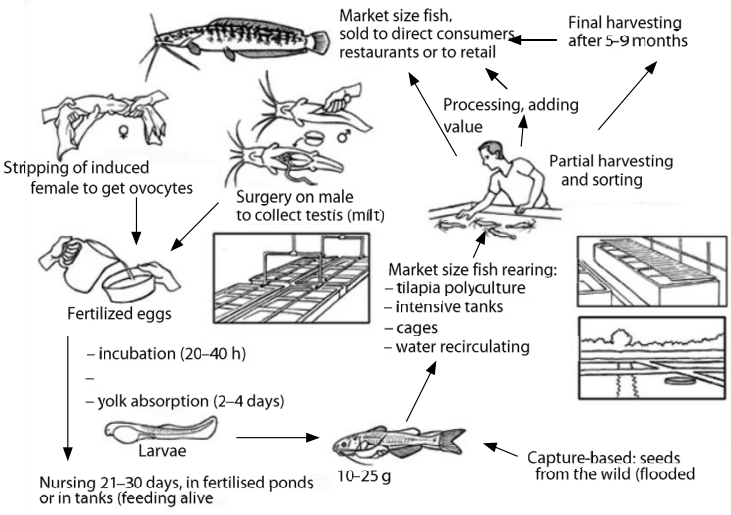

Fig. 7. *Production cycle (after Viveen et al., 1986).*

Successful hybridization between African sharptooth catfish, *Clarias gariepinus* (Burchell 1822) and vundu catfish, *Heterobranchus longifilis* (Valenciennes, 1840) (Hecht & Lublinkhof, 1985; Legendre *et al*., 1992). Legendre *et al.* (1992) found that these hybrids are viable and their survival is comparable to native species. The growth rate of vundu catfish and its hybrids with African sharptooth catfish is faster than the growth rate of pure line of African sharptooth catfish. However, neither vundu catfish nor hybrids with African sharptooth catfish are reared for commercial purposes in the Czech Republic. The next species of genus *Clarias* Philippine catfish, *Clarias batrachus* (Linnaeus, 1758) (Fig. 8) inhabits Southeast Asia. It is characterized by high variability in colour, including the frequent occurrence of albinos in natural conditions. However, it grows to a smaller size than African sharptooth catfish.

Fig. 8. *Philippines catfish – Clarias batrachus (Linneaus, 1758). Photo by M. Kořínek – www.biolib.cz*

2. AIM

The aim of this publication is to record the eleborated technological procedure of intensive farming in African sharptooth catfish in heated water, especially recirculating aquaculture systems in the Czech Republic. This includes breeding of broodstock, their artificial reproduction, manipulation with gametes, egg incubation, fry and market size fish rearing. The present technology also includes the results of testing the production efficiency of commercially produced feed with its impact on the product's quality (by using of these methods – yield, organoleptic assessment, basic chemical analysis of meat).

3. FACILITIES FOR AUTHENTICATION OF TECHNOLOGY

Authentication of technology takes place at three workplaces. Part of experiments focused on artificial reproduction, manipulation with gametes and fry rearing was carried out in the experimental hall of the Research Institute of Fish Culture and Hydrobiology, Faculty of Fisheries and Protection of Waters (hereinafter RIFCH FFPW USB) in Vodňany (Fig. 9). Some experiments relating to fry and market size fish rearing took place at a farm of the BaHa, Ltd. company in Mydlovary (Fig. 10). Most of the experiments on artificial reproduction, manipulation with gametes, fry rearing and feeding experiments took place in the aquarium room of the Institute of Aquaculture, Faculty of Fisheries and Protection of Waters (hereinafter IA FFPW USB) in České Budějovice (Fig. 11 and 28).

Intensive rearing of African sharptooth catfish (*Clarias gariepinus*)

Fig. 9. *Experimental hall of RIFCH FFPW USB in Vodňany. Photo by J. Kouřil*

Fig. 10. *Rearing hall of the farm in Mydlovary (currently, rented by BaHa, Ltd company). Photo by J. Kouřil*

4. DESCRIPTION OF TECHNOLOGY

4.1. Rearing of broodstock

4.1.1. Technological procedure

Broodstock were reared in two grey fiberglass square shaped tanks with rounded corners (volume: 500 litres, depth: 0.5 metres) connected in one recirculation aquaculture system where mechanical and biological purification of water was ensured by a combined cellular filter from the Alcedor, Ltd. company in Zliv. The tanks were covered with massive self-supporting plastic lids which covered two thirds of the surface. The remaining third was covered by a plexiglass lid with hinges. This part was covered by a few kilograms of objects, so that the reservoir was sufficiently secured against an escape of fish (Fig. 11).

Fig. 11. *Recirculation aquaculture system for rearing of African sharptooth catfish broodstock (aquarium room IA FFPW USB). Photo by B. Drozd*

Intensive rearing of African sharptooth catfish (*Clarias gariepinus*)

Both sexes (females and males) of an individual weight 1.0–5.5 kg were reared together at a stocking density of 50–150 kg.tank^{-1} (125–375 kg.m^{-3}) and fed mainly by food EFICO Alpha 717 6.0 (BioMar firm, Denmark). As supplementary food CatCo GROWER – 12 EF, CatCo GROWER – 13 EF, CatCo SELECT – 13 EF (Coppens firm, Netherlands) and possibly Harcsa- és Pisztráng nevelőtáp (Haltáp firm, Hungary) were used. Broodstock were fed in two or three daily doses. At the serving of food, fish usually respond violently, so fast covering of the tank is necessary to prevent water loss.

4.1.2. Results

Based on fish feeding activity, using the daily feed ration was verified in the range of 0.5–0.1% of the current biomass of fish. Before any manipulation with broodstock (even only at catching of fish), cleaning of the tank etc. feeding of fish had to be decreased for one to two days or completely omitted on the first day. In case of insufficient coverage of rearing tanks, fish can escape at any time (especially in the morning and during distraction of the fish). Therefore, the tanks have to be covered and burdened by heavy objects. Before harvesting of fish, it is necessary to reduce the water level in well covered tanks to a minimum. Then, it is possible to catch the fish. Otherwise, there is a risk of fish jumping out of the tank or possible injury.

Mortality of broodstock is exceptional. In most cases it is related to injuries caused by their jumping and falling, or in connection with the manipulation during artificial spawning, sorting etc. Individual annual weight gain of broodstock is about 1–2 kg. The largest broodstock (females) reaches an individual weight of 4–5.5 kg at the age of five years.

4.2. Hormonally induced artificial reproduction

4.2.1. Technological procedure

Artificial spawning of females was primarily focused on verification of using combined hormonal preparation Ovopel (Agrofish firm, Hungary) at different temperatures with the aim to determine the dependence of the latency interval on the water temperature. Broodstock from own breeding (see Chapter 4.1.) were selected after their capture from the tank. Before harvesting of the broodstock, the fish were not fed for one day. Females of 1–4 kg at the age of 1–3 years were artificially spawned. Selected females were separately placed into thermoboxes (volume: 20 liters) with aeration and soft inner wall preventing injury to the fish. After a few hours, the water temperature was modified to the desired values (19.1–31.5 °C). Simultaneously, three females were prepared for each individual artificial spawning at all temperatures. The water temperature in thermoboxes was recorded every four hours. Any temperature deviations were modified by exchanging small amounts of water (thanks to good insulating properties of thermoboxes the temperature deviations were minimal). Continuously, the thermoboxes with females were covered during the whole experiment, with their covers preventing potential attempts from escaping, jumping fish. From several hours to one-day adaptation to the desired temperature (based on temperature difference from the temperature at which broodstock was preciously bred), females were injected with a hormonal preparation.

Injection was performed in anaesthetized females. Fish anesthesia (Fig. 12) was induced by clove oil (Eugenol preparation, company Dr. Kulich Pharma Ltd., Hradec Králové) at a concentration of 0.06–0.10 ml.l^{-1} of water, an exposure time corresponded to the level of anaesthesia 2b (Hamáčková *et al.*, 2003). Temperature significantly affects the anaesthesia process (higher temperature lead to shortening of this interval) and the variability of individual susceptibility of fish to the used anaesthetics used. At a temperature of 23–25 °C, the level of anesthesia 2b occurs after 2–5 min.

Artificial stimulation of egg ovulation in African sharptooth catfish females was induced by the hormonal product Ovopel (AgroFish company, Hungary). This product is supplied in pressed white pellets. Each pellet contains two active ingredients: 20 µg synthetic GnRHa and 2 mg of dopamine inhibitor – metoclopramide. The recommended dose (Horvath *et al.*, 1997; Kouřil *et al.*, 2011) is one pellet per 1 kg of female weight for all species for which this prepatate is adequate. Pellets of the hormonal preparation Ovopel were stored in a plastic bottle with a waterproof lid in darkness at room temperature.

Intensive rearing of African sharptooth catfish (*Clarias gariepinus*)

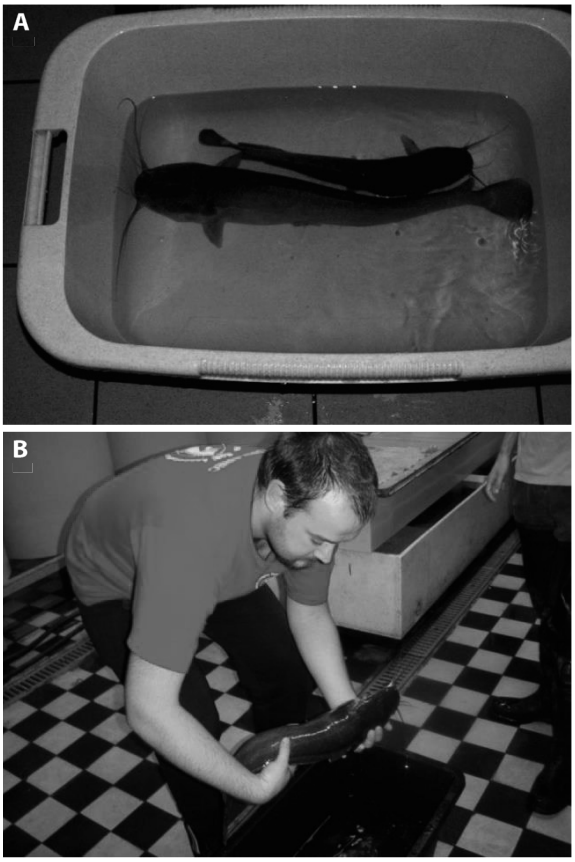

Fig. 12. *Anaesthesia (A) and manipulation with anaesthetized female (B) of African sharptooth catfish. Photo by J. Kouřil*

Before the female injection, the required number of pellets was collected. Then, pellets were crushed with a pestle in a dry mortar. Ovopel pellets are harder than the carp pituitary which is homogenized in a similar way. Therefore, greater power is necessary for crushing the pellets. Simultaneously, the mortar have to be covered (e.g. by food foil) to avoid loss of material during the crushing. After that, a physiological solution, which was stored in its original packaging (sterile packaged) in a refrigerator, was added to crushed pellets in a quantity corresponding to the dose and concentration required for stimulation of fish (Fig. 13). Homogenizing of the mixture

was carried out in a mortar with a pestle. Generally, the recommended dose of 1 pellet per 1 kg of female weight (at dilution: 1 pellet per 0.5 ml of physiological solution) was used. Immediately after that, the injection of females was performed. In the case, if the preparation of hormonal suspension was carried out a few hours in advance, the suspension was stored in a glass breaker (covered with aluminium foil) in a refrigerator at +5 °C. Suspension of the homogenized hormonal preparation was sucked into a disposable syringe in a required volume (with respect to the weight of the female) separately for each individual fish. Then, mortar and pestle were appropriately washed with hot water and dried, ready for further use.

Fig. 13. *Tools needed for preparation of suspension of hormonal treatment Ovopel for artificial ovulation in African sharptooth catfish. Photo by B. Drozd*
Top row (left to right): beaker, graduated cylinder, physiological solution (original packaging), middle row: pestle and mortar with pellets of hormonal preparation Ovopel; bottom row: syringes with needles (diameter 0.6 mm).

Subsequently, the females were taken out from the anaesthetic and an intramuscular injection was carried out (Fig. 14). Then, the fish were washed by water to remove residual anaesthetic from their surface and they were put back into thermoboxes. About 2 h before the expected ovulation, females were visually or by hand palpation controlled in half-hourly intervals. When the first eggs where found on

the walls or at the bottom of thermoboxes, the artificial spawning was immediately performed (comparison of female just before and after the artificial spawning – see Fig. 15). The time to ovulation or time of the artificial spawning, was recorded with an accuracy of fifteen minutes. The eggs were spawned (Fig. 16) into a dry container of a pre-known weight and marking. Each female was hand-stripped into a separate container (Fig. 17).

Fig. 14. *Injection into the dorsal muscle in African sharptooth catfish female. Photo by B. Drozd*

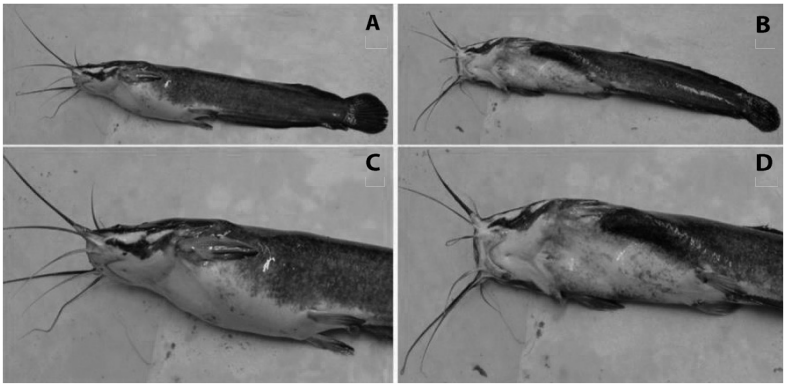

Fig. 15. *Ventrolateral view of African sharptooth catfish female just before the artificial spawning (A; C – detail of lateral part) and after spawning (B; D – detail of lateral part). Photo by B. Drozd*

Fig. 16. *Artificial spawning of African sharptooth catfish female. Photo by J. Matoušek*

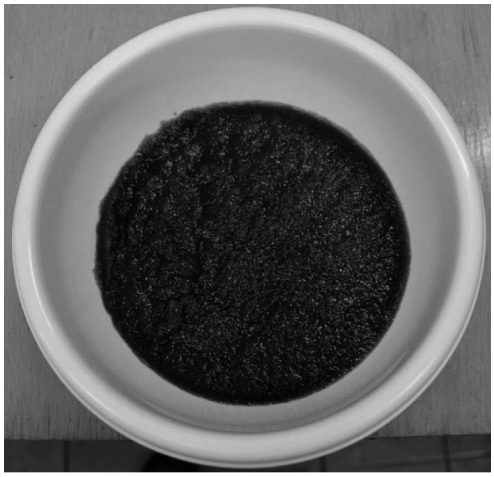

Fig. 17. *Freshly spawned eggs of African sharptooth catfish have greenish-yellow to greenish-brown colour. Photo by Foto B. Drozd*

Intensive rearing of African sharptooth catfish (*Clarias gariepinus*)

The abdominal area or other body parts of females were treated with a weak solution of hypermanganate (potassium permanganate concentration of: 3 grains per 500 ml of water). Then, the females were put back into thermoboxes. A water exchange was performed twice a day with a gradual transition back to the water temperature in the broodstock rearing tank. Due to control of the health condition of females, as well to prevent aggressiveness of just spawned fish in the broodstock tank, artificially spawned females were placed back into the broodstock tank after 2–3 days.

Sperm (milt) for egg fertilization was obtained from just killed males using the procedure introduced by Hamáčková *et al.* (2007). Usually, eggs were fertilized by a mixture of sperm from two males. Males were caught from the tank a few hours before the planned artificial spawning of females. They were placed separately into covered boxes. Just before the estimated time of ovulation and consequent artificial spawning of females, the males were taken out of the water. After their killing, the gonads (*testes*) were removed using surgical stainless steel scissors so as to avoid contamination of the gonads with water or blood (Fig. 18). Thereafter, the gonads (Fig. 19) were dried on a filter paper and put on a square of a dry inert technical textile (net with mesh size 0.5–1.0 mm) with an area of 25 x 25 cm. Male gonads were cut into approx. 1 cm pieces by use of dry stainless scissors (Fig. 20A). The textile was held above the glass or plastic container, in which the sperm was dripped. After that, the textile with cut male gonads was gently pressed by fingers from the outside and another portion of seminal fluid containing sperm dripped was obtained (Fig. 20B). The sperm was temporarily stored in a covered dish to prevent the contamination of water in the refrigerator +5 °C. During the next 10 min to 1 h the sperm was used for insemination of eggs.

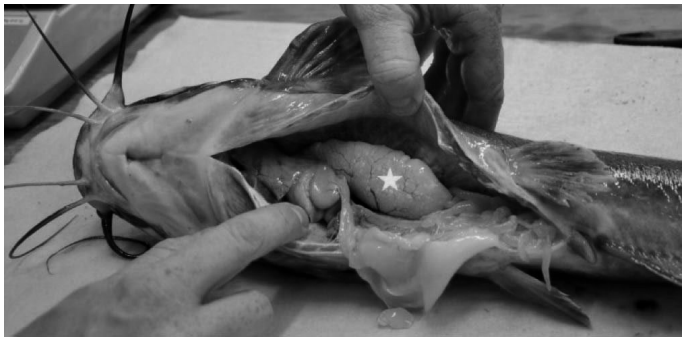

Fig. 18. *Dissection of gonads (testes; marked by a star) from the body cavity of African sharptooth catfish males. Photo by J. Matoušek*

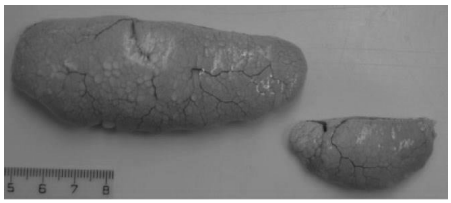

Fig. 19. *Dissected gonads (testes) of African sharptooth catfish. Photo by B. Drozd*

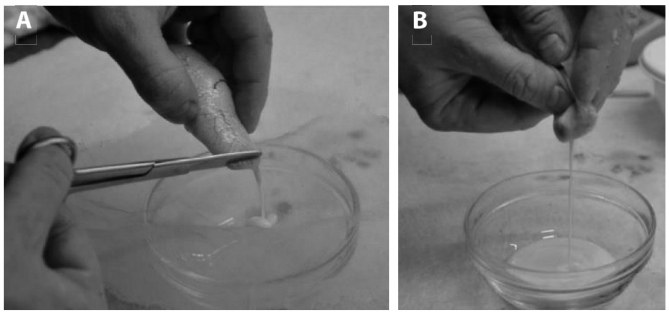

Fig. 20. *Obtaining of the sperm for egg fertilization by cutting (A) and pushing through an inert technical textile (B) of dissected gonads of African sharptooth catfish male. Photo by J. Matoušek*

Egg insemination and fertilization (Fig. 21) were performed according to procedure presented by Adamek (2001) and Hamáčková *et al*. (2007). In the short term, spawned eggs were kept in containers covered by wet, properly wrung out textile which did not touch the eggs. Containers with unfertilized eggs were placed on the floor (temperature of eggs was about 20 °C). Before insemination, the eggs were stored for 10 min. to 1 hour. Before egg insemination, the eggs were divided into 200–300 g portions into separate containers. Egg fertilization was carried out with 2-5 ml of sperm (Fig. 21A). Then, the mixture of gametes was slightly mixed with a dry plastic or rubber spatula. This was followed by the fertilization of eggs, which was performed by pouring of water until the gamete mixture was completely under water and about 0.5 – 1 cm layer of water was above the surface. (Fig. 21B). The resulting mixture was stirred approximately 1 min. Then, the container with sex products and water was left for 2 min at rest. In the next 2-4 minutes, the fertilized eggs were washed with water. By repeatedly mixing and pouring of water on the eggs, the elimination of sperm residues or infrequently occurring white (fertilization unable) eggs was implemented. After that, the external adhesive layer of eggs was activated. Therefore, the eggs were immediately poured on incubation sieves which were submerged under water and well attached to the wall of the plastic trays. This activity was carried out as quickly as

possible and in such a way that the eggs were well spread over the whole surface of the textile to prevent their greater accumulation and subsequent formation of lumps and clumps of aggregated eggs stuck together. Technical (inert) textile Uhelon of 0.5 mm mesh size proved to be the best for production of incubation sieves.

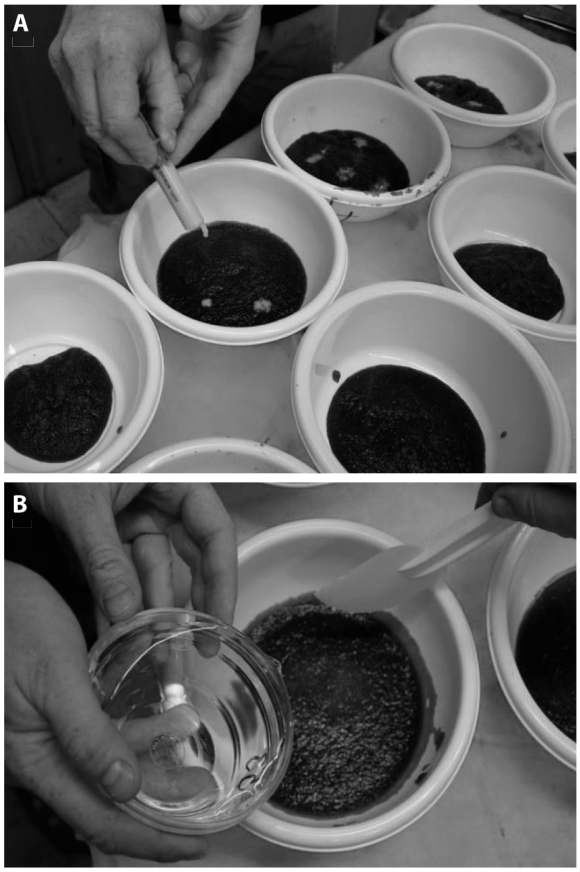

Fig. 21. *Egg insemination by sperm (A) and egg fertilization (B) of African sharptooth catfish. Photo by J. Matoušek*

An incubation trough (Fig. 22), or several throughs, was/were part of a separate recirculation system with a plastic reservoir tank under it/them. From the incubation tank/s, the water gravitationally spilled down into the reservoir tank/s and then the water was pumped up into a plastic pipe using a submerged pump. An aquarium UV lamp proved to be a useful part of pressure pipe/s that brought the water to the incubation trough (troughs). Perfect purity of all used components was necessary for the prevention of any water contamination during egg incubation.

4.2.2. Results

Generally, 40 females were injected during 10 separate experiments. Each experiment was carried out at different water temperatures in a range of 19.1–31.5 °C. Ovulation was induced and artificial spawning was performed in a total of 39 females (achieved success of 97.5% – see Tab. 4).

Tab. 4. *Length of latency interval and number of injected and ovulated (artificially spawned) females of African sharptooth catfish at different temperatures in the range of 19.1–31.5 °C.*

Temperature (°C)	Length of latency interval (h)	Number of fish	
		injected	ovulated
19.1	26.75	4	4
19.5	26.75	4	4
21.4	19.00	4	4
21.5	18.75	4	4
22.5	17.25	4	4
23.7	15.75	4	3
25.4	14.00	4	4
27.0	11.75	4	4
29.7	9.25	4	4
31.5	7.00	4	4

A comparison between the lengths of latency interval after using of Ovopel and carp pituitary preparation dependent on a water temperature of 18–32 °C is recorded in Tab. 5 (for use in hatchery practice). The latency interval length induced by Ovopel was observed by the inventors of this technology. The data from Tab. 4 were adjusted

by extrapolation and interpolation with subsequent rounding of numbers. Data regarding use of carp pituitary extract were taken from Adamek (2001).

Tab. 5. *Dependence of latency interval length (h) on water temperature at hormonal induction of ovulation in African sharptooth catfish females using a single injection of Ovopel (data observed by authors of this technology) in comparison with a single injection of carp pituitary extract – CPE (results published by Adamek, 2001).*

Temperature °C	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
Ovopel (h)	–	27	23	20	18	16	15	14	13	12	11	10	9	8	7
CPE (h)	21	–	–	19	18	15	13	12	11	10	9	8	7,5	7	–

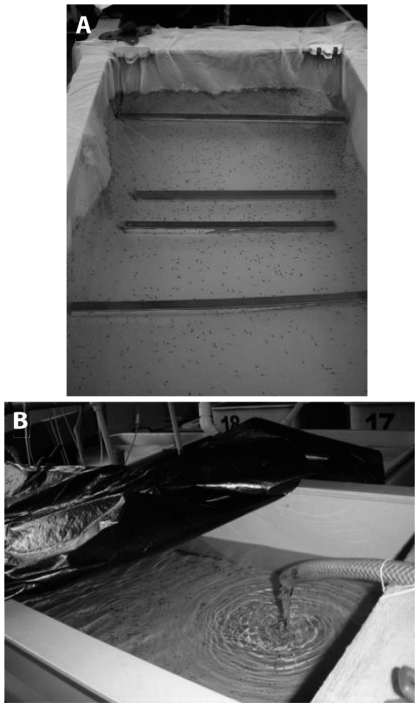

Fig. 22. *Trough for incubation of eggs and newly hatched fry of African sharptooth catfish. Photo by J. Kouřil. A: Trough with yolk-feeding larvae; B: Inlet part of the trough with externally fed larvae (overshadowing of tank).*

4.3. Temperature effect on storage of artificially spawned eggs before fertilization

4.3.1. Technological procedure

In total, three females were artificially spawned and their eggs were used for this experiment. After the Ovopel injection, the females (see Chapter 4.2.) were kept until artificial spawning (during latency period) in a water temperature of 23.7 °C. From each female, a part of the spawned eggs (about 200 g) was immediately taken to an environment at a different temperature. The containers with eggs were covered with wet, wrung out textile and separately stored in thermoboxes (Fig. 23). There was a temperate (required) water bath at the bottom of thermoboxes. Different temperatures were monitored and maintained (through the addition of ice cubes, or a small amount of warm water) at 5, 10, 15, 20, 25 and 30 °C (in compliance with maximum deviations of 0.1 °C from desired value) in thermoboxes. Gradually, samples of eggs stored at various temperatures were removed with a dry plastic spoon after ¼, ½, 1, 2, 4, 6 and 8 h from spawning, respectively. Then, these egg samples (about 100 eggs per one sample; always three replicates for each time interval), including a sample collected immediately after the spawning, were placed into a dry glass container with a temperature of 25 °C. Furthermore, the eggs were inseminated with sperm mixture from two males and after that they were fertilized by pouring of water at an identical temperature (25 °C). Subsequently, the mixture of eggs and sperm was stirred with a plastic spoon so that unstuck eggs spread over the surface of the container. After 10 min of fertilization, the water from the container with fertilized eggs was repeatedly decanted and poured. Eggs were washed with clean water to eliminate the remnants of sperm and ovarian fluid. Containers with fertilized eggs were placed together on a table at an air temperature of 25 °C (Fig. 24). During egg incubation, water was twice exchanged in containers. After 12 hours, fertilized (developing) and white (unfertilized, or none developing) eggs were counted. Based on these data, the fertilization rate of eggs was determined in %.

Intensive rearing of African sharptooth catfish (*Clarias gariepinus*)

Fig. 23. *Storage of artificially spawned eggs of African sharptooth catfish in a thermobox. Photo by J. Kouřil*

4.3.2. Results

During the experiment, a significant effect of different temperatures on the length of fertilization capability was found out in African sharptooth catfish. In control groups (eggs fertilized immediately after spawning), an average fertilization rate of about 90% was achieved. For storage of just spawned unfertilized eggs of African sharptooth catfish, a temperature range between 15 and 20 °C can be considered (see Tab. 6). After 6 hours of spawning, the fertilization rate of eggs still reached about 75% and more (*note: this fertilization rate was chosen by the authors as arbitrary limit for spawning which can be regarded as successful from a hatchery point of view). At lower temperatures, the fertilization ability decreases significantly faster. Already after 1.5 hours, the drop of egg fertilization below 75% was observed at 10 °C. After 0.5 hour, the fertilization rate decreased after the limit mentioned at 5 °C. On the contrary, a lower decline of fertilization ability was found out at higher temperatures. After 4 h, the fertilization rate of eggs was above 75% at 25 °C. After 2 hours, the mentioned arbitrary limit of fertilization rate was reached also at a temperature of 30 °C. Based on the results, temperatures of 15–20 °C (or even 25 °C) can be recommended for storage of spawned unfertilized (uncontaminated with water) eggs of African sharptooth catfish up to 4 h (at temperature of 15 and 20 °C up to 6 h). At these temperatures and time intervals, no significant reduction (decrease below 75%) of fertilization ability was observed.

Tab. 6. *Temperature effect (°C) and storage length (h) of unfertilized eggs of African sharptooth catfish without water contamination on their fertilization ability (%).*

Temperature (°C)	Length of egg storage (h)							
	0.5	**1**	**1.5**	**2**	**3**	**4**	**6**	**8**
5	72	68	65	48	51	16	24	9
10	***79***	***85***	68	71	63	54	43	6
15	***82***	***85***	***77***	***78***	***77***	***77***	***78***	37
20	***90***	***84***	***85***	***77***	***90***	***81***	***75***	29
25	***84***	***91***	***84***	***84***	***75***	***76***	68	19
30	***83***	***84***	***82***	***79***	53	33	1	0

* ***Bold and italic*** are the combinations of temperature and storage length, in which the egg fertilization rate of 75% and more was reached.

Intensive rearing of African sharptooth catfish (*Clarias gariepinus*)

Fig. 24. *Incubation of African sharptooth catfish eggs in glass containers during experiments focused on study of the environment temperature effect on the storage of artificially spawned eggs before insemination and the length of the water contamination effect on the rate of micropyle closure. Photo by J. Kouřil*

4.4. Effect of length of water contamination of eggs on the rate of micropyle closure

4.4.1. Technological procedure

Generally, two indicative experiments on the indirect determination of the time interval of unfertilized egg micropyle closure after contact with water were carried out. Artificially spawned eggs from three or four females were used during the first and second experiment, respectively. After the Ovopel injection (see Chapter 4.2.), females were kept at 23–24 °C until the spawning (during latency period). Always, an amount of around 100 eggs (from each female) was placed into every marked glass container using a plastic spoon. Overall 20 experimental variants were realized (see below) and one control with three containers was always used for each variant during all experiments. Immediately after spawning, the eggs were inseminated and then activated by water (in a close time interval) in all control groups. Water was added to the eggs in containers at 30 second intervals. Subsequently, a mixture of fresh sperm (from two males) was added to each container using a syringe at 1-minute intervals. Immediately after the addition of sperm, the mixture of gametes and water was gently mixed. This procedure was used in each of the experiments. In total, 20 variants with different water contamination lengths (0.5–10 min. before sperm addition) of eggs were performed. Like in the experiment mentioned in Chapter 4.3., water from every container was repeatedly decanted and poured after 10 min. from sperm addition. The eggs were always washed with clean water to eliminate sperm and ovarian fluid remnants. Similarly, the fertilization rate of eggs was determined after 12 h from the beginning of the experiment. During egg incubation (Fig. 24), all containers were placed on a table in a room with a 25 °C air temperature. During incubation, water was exchanged twice. After 12 h from hatching, the egg hatchability was evaluated in %.

4.4.2. Results

In the control groups (sperm was added to eggs earlier than water), egg fertilization fluctuated in repetitions (n = 7) between 43–95% (75.13 ± 20.21%; mean ± S.D.) and fry hatchability fluctuated between 20–71% (47.00 ± 19.93%). During water contamination of eggs (at indirect micropyle closure after contact with water), very rapid reductions of the egg fertilization rate and subsequent hatching rate were observed with an increasing length of presence of eggs in water (see Fig. 25). Already after 1 min. from water contamination, egg fertilization rate and hatching rate decreased to half. After 2 min. of water contamination, both parameters decreased to a quarter of values that were recorded in the control group. After 3 min. from water contamination, the fertilization rate decreased below 10%. In connection with this, also

the hatching rate decreased. From 2.5–3 min. (and more), no hatchlings were recorded (the hatching rate dropped to 0%).

Based on this indicative experiment, it can be concluded that water contamination of African sharptooth catfish eggs (e.g. at inadequately professionally controlled artificial spawning) has an adverse effect on egg fertilization and the hatching rate. This effect increases with an increasing length of presence of eggs in the water without sperm contamination. This effect can be prevented through perfect protection from water contamination of eggs (e.g. well dried abdominal area of female and safe storage of spawned eggs – covering with carefully wrung out textile). Nevertheless, if water contamination will occur during or after the spawning, this problem can be eliminated with immediate egg insemination (to 1 min. from contact of eggs with water) by sperm and subsequent activation with water (sperm and water have to be prepared in advance), but without a guarantee of sure success. Risk of water contamination of eggs may be reduced by separate spawning of eggs from each female into different dry containers.

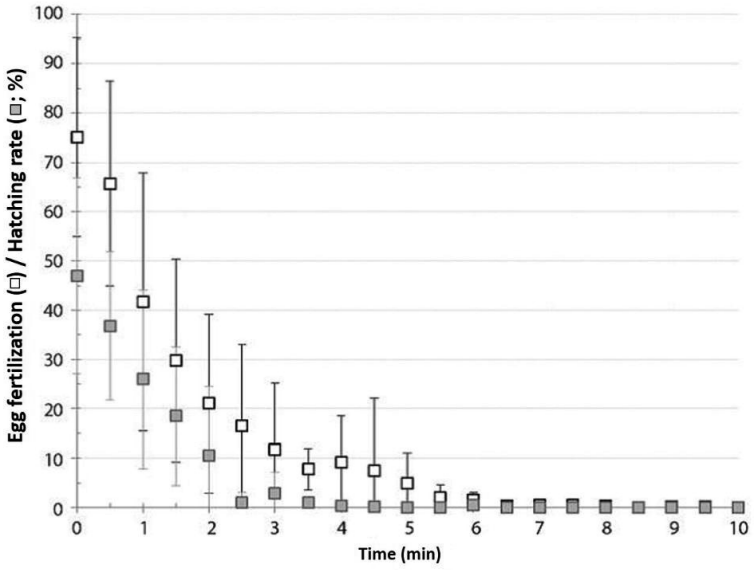

Fig. 25. *Fertilization and hatching rate (mean ± S. D.; %) of African sharptooth catfish eggs in dependence on time interval length of water contamination of unfertilized eggs till their fertilization (addition of sperm).*
□ *Fertilization rate* ■ *Hatching rate*

4.5. Effect of temperature and egg incubation on hatching and early development

4.5.1. Technological procedure

A series of experiments was aimed at observing the effect of water temperature on ontogenetic development: the length of incubation period, hatching period, onset of intake of external feeding, size and survival of African catfish larvae. Experiments were carried out under laboratory conditions in non-flow aquariums and glass containers placed in temperate baths where the temperature was continuously monitored and regulated using thermostats and electric heaters (range: 19–33 °C, tolerated deviation: maximum 0.1 °C from required temperature.

4.5.2. Results

It was observed that the length of the incubation period (interval from egg fertilization to hatching) and hatching period (interval from start of hatching until the end of hatching) is dependent on the water temperature. Both parameters decreased with increasing water temperature (see Tab. 7). At optimal temperatures (19–31 °C), the length of incubation and hatching period was 19–39 h and 3–5 h, respectively.

Size (total body length and wet weight) of freshly hatched individuals called larvae (in the interval from hatching until full yolk absorption) was dependent on the water temperature ranging from 23–33.5 °C. The size and developmental stage of larvae decreased with increasing water temperatures, because just hatched individuals which were incubated at higher temperatures reached a lower size and ontogenetic stage, than the fish hatched at lower temperatures. Freshly hatched larvae of African sharptooth catfish reared at optimal temperature conditions (23–30 °C) were 4–5 mm long and weighed 1.2–1.6 mg. The yolk volume of just hatched larvae correlated with the size of spawned eggs (yolk volume of hatched individuals increased with rising egg size). At optimum water temperatures (23–30 °C), yolk volume achieved 0.8–2 µl.

Tab. 7. *Length of incubation period (interval from fertilization until hatching; indicated for moment of hatching of 50% of individuals; h) and hatching period (interval from beginning until the end of hatching; h) depending on the water temperature (°C) in African sharptooth catfish.*

Water temperature (°C)	19	21	23	25	27	29	31	33
Length of incubation period (h)	70	48	39	29	24	20	18	16
Length of hatching period (h)	2	6	5	4	3.8	3	2.8	1.6

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Generally, African sharptooth catfish are characterized by highly variable and often quite low fertilization rates and subsequent hatching rates (i.e. the number of surviving individuals at the time of hatching). Within an optimum temperature range (23–30 °C), the hatching rate reached to 95%. However, significantly lower values could be achieved dependending on the spawning conditions, broodstock quality and professional experience. On average, hatching rate about 50–70% can be achieved, but values of about 25% are not exceptional (see Tab. 8).

Tab. 8. *Hatching rate (% of hatched larvae from the total number of controlled fertilized eggs) depending on the water temperature (°C) in African sharptooth catfish.*
Data in brackets represent the minimum and maximum average values achieved during different spawnings in 2009–2011.

Water temperature (°C)	Hatching rate (%)
18	0
19	29
21	75 (58; 91)
23	73
24	81 (70; 95)
25	78 (25; 88)
27	64 (40; 77)
29	35 (34; 37)
30	28 (25; 42)
31	14 (13; 21)
33	11 (10; 18)
35	0

The length of endogenous feeding period, i.e. time from hatching until the onset of external food intake (mixed feeding period), was dependent on the incubation temperature. It decreased with an increasing water temperature (see Tab. 9). Under optimal temperature conditions (23–30 °C), larvae of African sharptooth catfish had startedan intake of external food after about 40–80 h from hatching at size of 6–8 mm and wet weight 2–4.5 mg.

Tab. 9. *Length of endogenous feeding period (i.e. from hatching until the onset of intake of external food; mean, h) depending on water temperature (°C) in African sharptooth catfish.*

Water temperature (°C)	21	23	25	27	29	31
Length of endogenous feeding period (h)	93	77	65	50	43	34

The length of the yolk absorption period (i.e. from hatching until the full yolk absorption when the larvae completely resorbed their yolk reserves and they started to use only external food as the only energy source), was dependent on the water temperature. This parameter decreased with increasing water temperature. Under optimal temperatures (23–30 °C) and feeding conditions (*ad libitum* feeding), full yolk absorption occurred. The transition to only external feeding averaged about 10–26 days from hatching (see Tab. 10). If the larvae were not fed with external food, a depletion of yolk reserves occured after 2.1–4.6 days from hatching (*these data serve as information to select the suitable time for the beginning of brine shrimp nauplii or starter feed application*).

Tab. 10. *Length of period from hatching until full yolk absorption (mean, days) in dependence on water temperature (°C) in fed and unfed larvae of African sharptooth catfish.*

Water temperature (°C)	21	23	25	27	29	31
Length of yolk absorption period in *fed* larvae (days)	33.8	25.8	20.4	15.4	12.5	9.0
Length of yolk absorption period in *unfed* larvae (days)	6.8	4.6	4.0	2.7	2.2	2.0

After full yolk absorption, the size of fed larvae (total body length and wet weight), which were kept without external food, did not differ statistically in a thermal range of 23–30 °C (it was not dependent on the water temperature). At optimum water temperatures (23–30 °C), fed larvae were 6.5–7.5 mm long and weighed 2–3 mg. However, the size of fed larvae significantly varied in dependence on the water temperature after full yolk absorption. In this case, size exhibited inversely proportional dependence on the incubation temperature (it decreased with increasing temperature). Larvae reared at optimal temperature conditions were 10–20 mm long (about doubled in comparison with experiments without external feeding) and weighed 15–50 mg.

The size of fed larvae (total body length and wet weight) was temperature dependent after full transition to exogenous feeding (yolk was fully absorbed). Larval body length and weight decreased with increasing water temperature and averaged

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between 10–20 mm and 15–50 mg, respectively (*body length of unfed larvae reached 6.5–7.5 mm and weighed 2–3 mg*).

The survival rate after hatching (interval from hatching until the full yolk absorption) ranged up to 75% at optimal temperatures, but on average it was 50% (see Tab. 11).

Tab. 11. *Average survival rate after hatching (interval from hatching until the transition to exogenous feeding/ full yolk absorption; %) based on water temperature (°C) in African sharptooth catfish.*

Water temperature (°C)	Survival rate (%)
21	16
23	48
25	48
27	51
29	47
30	52
33	12

Fry should be reared at a water thermal range of 27–30 °C in shallow flow-water troughs with brine shrimp nauplii - *Artemia salina* (Linnaeus, 1758) initial feeding with subsequent transition (after several days) to starter feeding (Fig. 26 and 27). The condition of rearing success is closely related to high purity of the environment and good water quality.

Fig. 26. *Rearing of African sharptooth catfish fry. Photo by J. Kouřil*

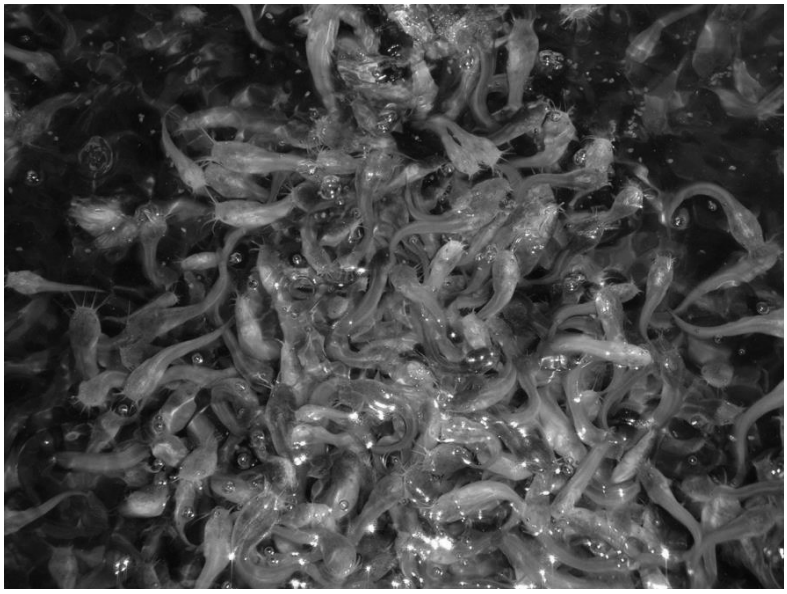

Fig. 27. *Detail of African sharptooth catfish fry at external feeding by starter food. Photo by B. Drozd*

4.6. Testing of feed for breeding fish in market size

4.6.1. Technological procedure

Generally, three consecutive feeding tests were carried out on African sharptooth catfish in market size. Overall, 10 different commercially produced feeds were tested as potentially applicable to African sharptooth catfish. Some of them were directly produced for catfishes (African catfish). Several feeds were tested only once, others repeatedly.

Fish fry with an average weight of 200–300 g were used in feeding tests. Fry was reared in the same type of tanks and fed with EFICO Alpha 714 feeding (BioMar company, Denmark) before the experiments. Each test started with a three-week adaptation period. At the beginning of the test, the tanks were harvested and current stocking densities were joined in one reservoir tank and then the same numbers of fish were put into all experimental tanks under following principles: individual weight (with

accuracy of 1 g), sex determination (half-representation of both sex in each tank), well developed fish without cannibalism or damage, relatively low size variability (less than 1% of the average biomass) and about the same total weight (biomass) of fish stocks. In the first three-week period, the fish were getting used to the tested food which they received during the experimental period. The Daily food ration (DFR) corresponded to this adaptation. The first day after stocking, the fish were fed with one quarter of DFR. The daily food ration was gradually increased to the recommended amount of DFR. Based on the feed, the adaptation lasted from a few days to 1–2 weeks. Then, the fish began to feed in required quantity (full amount of DFR) with respect to the average individual weight and current stocking biomass. Daily food rations were divided into six daily doses (at 8, 10, 12, 15, 18 and 20 h). The relative amount of DFR (in relation to estimated current biomass) was determined in advance, according to the recommended DFR. Alternatively, DFR was corrected according to eaten amount of feeding in the previous day. There was also an effort to follow the same DFR for all experimental groups. However, if apparent differences were observed in acceptance of various feeds, the principles have had to be gradually changed. For these reasons, relative DFRs were slightly diversified. Current DFR was calculated according to actual stocking biomass. On the following day, relative DFR was determined (in % stocking biomass) and accordingly the absolute DFR was calculated (in grams) for each tank. If the full amount of DFR was eaten by the fish, the weight of feed was added to the original weight of biomass and then the theoretical stocking biomass was calculated for the next day. If the DFR was not eaten by fish during one day, the weight of the remaining feed was deducted from DFR and only the real amount of eaten feed was recorded. Rarely, the increase of the DFR was performed when the fish exhibited a higher willingness to accept the feed. Usually, it occurred after the day when incomplete DFR was served to fish for any reason. In that case, determination of absolute DFR was carried out in the opposite way. The determined DFR had to be increased even more by the weight of unconsumed feed. This procedure was followed throughout all 20 feeding days. The following day (21st experimental day), the harvesting of stocks, individual weight and sex determination of all the fish were performed. The fish were re-stocked into the experimental tanks. Before and after the harvesting day, the fish were not fed.

After the twenty-day feeding period, a weight change of the fish biomass was found in all the stocks (the difference between the stocked and harvested fish weight in grams, called also absolute weight gain). From this value and the average between the stocked and harvested biomass, the relative daily gain of biomass (in %.d^{-1}). Also, a total absolute weight of feeds actually eaten was determined after the twenty-day feeding period. From the absolute gain of fish biomass and real feed consumption, the food conversion ratio (FCR) was calculated. It expresses the feed consumption per unit of weight gain, e.g. kilograms.

Formula for calculating of FCR: $$FCR = \frac{F}{(W_t - W_o)}$$

F – feed consumption during the tested period
W_t – stocking weight at the end of experiment
W_o – stocking weight at the beginning of experiment

The real relative DFR was calculated from average stocked biomass, harvested biomass and absolute DFR. Real relative DFR was used for the determination of initial relative DFR for the following sub-feeding period. Based on price, absolute weight of eaten feed and absolute weight gain of fish biomass, the costs of one kilogram weight gain of biomass were calculated in selected perspective feeds. Cost determination was carried out summarily for the entire rearing period (for period of several partial twenty-day tests). Feed prices used in the cost calculation are based on retail prices for small farmers (i.e. customers with a consumption of 20–25 kilograms bags of feed per month.

The first feeding experiment took place in aquariums placed in a two-decker metal stand (Fig. 28A). In the recirculation system, the continuous water flow and outflow was maintained in aquariums. The recirculation system consisted of a sedimentation tank (placed on the floor under a stand) and upper tank with a submerged biological filter (placed at the top, above the experimental aquariums). Aeration was installed into the aquariums. Water flow ensured water exchange about once every 1–2 hours. The aquarium walls and glass were cleaned with a foam sponge in every day. Sludge was extracted twice during rearing and once at fish harvesting. The sedimentation tank and biological filter were purified as needed; they were cleaned more frequently at the end of rearing in relation to increasing biomass and pollution.

The next two feeding experiments took place in cylindrical tanks from white plastic material with usable volume of 315 l (Fig. 28B), upper inlet and lower outlet of water. The water outlet flowed into a sedimentation tank, then the water was pumped about two meters above into shallow supply tank, from where it uniformly flowed into the trickle biological filter. Under the biological filter, the water was trapped into the collecting tank and then gravitationally flowed through the pipe into other tanks. Aeration was installed into each tank. Water flow ensured the exchange of water about once per every 3–4 hours. Each week, the tank walls were cleaned using a foam sponge (twice a rearing and once a fish harvesting).The sedimentation tank and biological filter were purified as needed, they were cleaned more frequently at the end of rearing in relation to increasing biomass and pollution.

Intensive rearing of African sharptooth catfish (*Clarias gariepinus*)

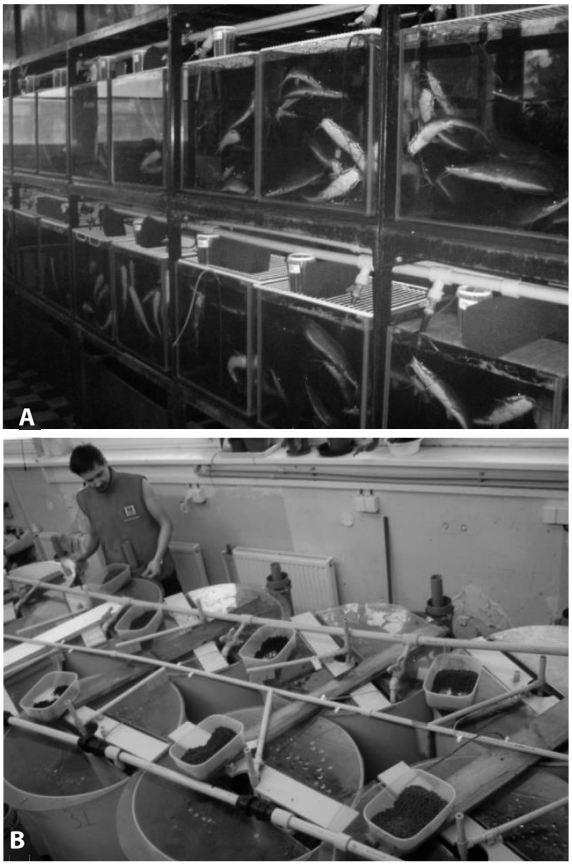

Fig. 28. *Interior of aquarium room of the Institute of Aquaculture FFPW USB in České Budějovice: experimental aquariums (A) and circular tanks (B) in recirculating aquaculture systems with course of feeding tests in African sharptooth catfish. Photo by J. Kouřil*

Both systems were refilled daily with warm tap water. The water temperature in recirculating aquaculture systems was maintained thanks to sufficient heating of the aquarium room. When necessary, the desired water temperature was achieved using electrical heaters. African sharptooth catfishes were reared to market size (a total individual body weight of 800–1 500 g).

In the first experiment, in total six different feeds (in three repetitions) were tested in 18 aquariums. Five salmonid feeds – Aqua Focus (Aller, Poland), EFICO Alpha 714

(BioMar, Denmark), Skretting F-2P B40 (Skretting, Norway), Troco Supreme-22 a Troco Prime-18 (Coppens, Netherlands) and one feed for trouts and African sharptooth catfishes – Harcsa-és Pisztráng nevelõtáp (Haltáp, Hungary) were tested. The specification of individual tested feeds is listed in Tab. 12. During the first experiment, one adaptation and four experimental feeding periods were realized. The total length of the experimental feeding test lasted about 84 days. The average water temperature was 26.0 °C.

Tab. 12. *Specifications of tested feeds used during the first feeding experiment in African sharptooth catfish (feed from Haltáp company was used also during the third feeding experiment).*

Feed	Aqua Focus	EFICO Alpha 714	Skretting F-2P B40	Troco Supreme-22	Troco Prime-18	Haltáp
Granule size (mm)	4.5	4.5	4	4.5	4.5	5
Protein content (%)	37	42–46	41	44	42	48
Fat content (%)	12	13–16	12	22	18	6.4
Ash content (%)	7	6.4	6.5–8	7.1	7.1	
Fiber content (%)	4	6	2.5–3	1.8	2.9	1.8
N content in dry matter (%)	6.5					
P content in dry matter (%)	1.2	1	0.85–1.4	0.9	1	1.3
Ca content in dry matter (%)		0.86		1.3	1	1.4
Na content in dry matter (%)				0.3	0.2	0,3
Mn content in dry matter (mg.kg^{-1})		30				
Cu content in dry matter (mg.kg^{-1})			6			
Amino acid content (%)			2.5			6
Vitamin A (IU.kg^{-1})	2 500		5 000	10 000	10 000	1 400
Vitamin D3 (IU.kg^{-1})	500			3 000	2 000	140
Vitamin E (mg.kg^{-1})	150		150	200	150	70
Gross energy (ths kJ.g^{-1})	19.5	20–22		22.4	21.4	
Digestible energy (ths kJ.g^{-1})	15.3	15.5	17.6	20	19.2	
Price (CZK.kg^{-1})	31	35	37	42	45	32

*** IU** means ***international units***

Intensive rearing of African sharptooth catfish (*Clarias gariepinus*)

In the second experiment, in total four feeds were tested in 12 tanks (each feed in three repetitions). Three feeds for African sharptooth catfish keříčkovce – CatCo GROWER – 12 EF, CatCo GROWER – 13 EF and CatCo SELECT – 13 EF (Coppens, Netherlands) and one salmonid feed – Dibaq Trout Evolution (Dibaq, Spain) were tested. Specifications of the tested feeds are listed in Tab. 13. During the second experiment, one adaptation and four feeding periods were carried out. The length of the experimental feeding test lasted 84 days. The average water temperature was 26.0 °C.

Tab. 13. *Specifications of tested feeds used during the second feeding experiment in African sharptooth catfish (feeds CatCo GROWER – 12 EF, CatCo SELECT – 13 EF were used also during the third feeding experiment).*

Feeds	CatCo gROWER-12 EF	CatCo gROWER-13 EF	CatCo SElECt-13 EF	Dibaq trout Evolution
Granule size (mm)	4.5	4.5	4.5	5
Protein content (%)	45	42	42	38–40
Fat content (%)	12	13	13	24
Ash content (%)	8.7	7.4	8.5	8.5
Fiber content (%)	1.9	2.7	1.9	1.8–2.2
P content in dry matter (%)	1.1	1	1.1	0.85
Cu content in dry matter ($mg.kg^{-1}$)				7
Vitamin A ($IU.kg^{-1}$)	10 000	10 000	10 000	7 500
Vitamin D3 ($IU.kg^{-1}$)	2 000	2 000	2 000	1 000
Vitamin E ($mg.kg^{-1}$)	200	200	200	150
Gross energy ($kJ.g^{-1}$)	19.9	20.2	20.0	
Digestible energy ($kJ.g^{-1}$)	18.1	18.1	18.1	
Price ($CZK.kg^{-1}$)	51	51	51	48

*** IU** means ***international units***

In the third experiment, three different feeds were tested in nine tanks (each feed in three repetitions). They were special feeds produced for rearing of catfishes. They were tested already in two previous experiments – CatCo GROWER – 12 EF (Fig. 29A), CatCo SELECT – 13 EF (Fig. 29B; Coppens, Netherlands) and Harcsa-és Pisztráng

nevelõtáp (Haltáp, Hungary; Fig. 29C). During the third experiment one adaptation and three feeding periods were realized. The total length of the feeding experiment was 63 days. The average temperature was 24.5 °C.

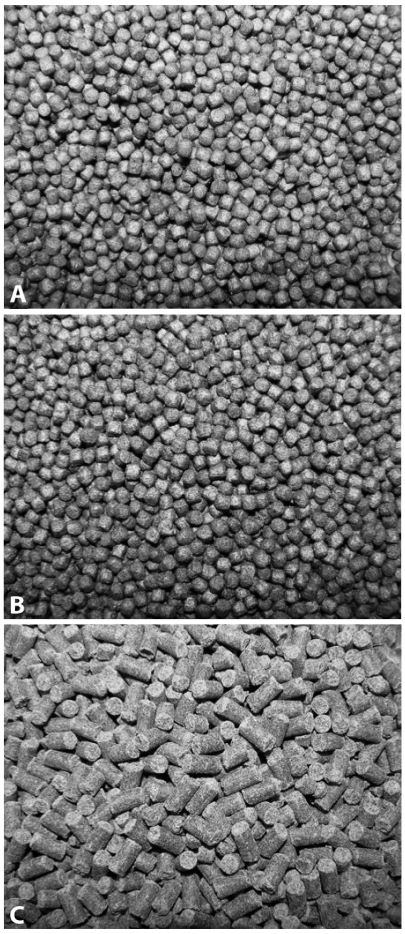

Fig. 29. *Special tested feeds for catfishes were from the Dutch company Coopens: CatCo GROWER – 12 EF (A), CatCo SELECT – 13 EF (B) cnd Hungarian company Haltáp: Harcsa- és Pisztráng nevelõtáp (C) at production of African sharptooth market size catfish in market size during the third feeding experiment. Photo by O. Houda*

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4.6.2. Results

In the first experiment, the best feed conversion ratio (FCR) was reached in both feeds intended for salmonids from the Coopens company – Troco Supreme-22 (FCR = 1.19) and Troco Prime-18 (FCR = 1.26). The third best feed conversion ratio was observed in feed developed for salmonids and African sharptooth catfish from the Haltáp company – Harcsa-és Pisztráng nevelõtáp (FCR = 1.45), followed by a feed for salmonids from Aller company – Aqua Focus (FCR = 1.58) and the Skretting company – Skretting F-2P B40 (FCR = 1.74). At the same time, the highest increase of biomass was recorded in feed from the Haltáp company. The highest feed conversion ratio was surprisingly achieved in EFICO Alpha 714 feed from BioMar company (FCR = 1.97).

Then, the costs of one kilogram weight gain were observed in relation to using of various feeds. The lowest cost was reached in feed from Haltáp company (45 CZK.kg^{-1}), followed by Aqua Focus feeds (46 CZK.kg^{-1}) and TROCO SUPREME-22 (50 CZK.kg^{-1}). In other feeds the costs significantly exceeded 50 CZK.kg^{-1}, which can be taken as arbitrary limit of profitability of African sharptooth catfish rearing in RAS under small-farm conditions. Costs for other feeds were: Troco Prime-18 (56 CZK.kg^{-1}), Skretting F-2P B40 (57 CZK.kg^{-1}) and EFICO Alpha 714 (66 CZK.kg^{-1}).

In the second feeding experiment, the lowest feed conversion ratio achieved in Dutch feed developed for African catfish rearing from Coppens company – CatCo SELECT-13 EF (FCR = 0.85). Simultaneously, this feed produced the highest specific growth rate (1.30%). This led also to the lowest cost per kilogram of weight gain (43 CZK. kg^{-1}).

In the third feeding experiment, the most favourable feed conversion ratio was achieved with using of CatCo feed GROWER-12 EF (FCR = 0.82). Followed by previously proven feed – CatCo SELECT-13 EF (FCR = 0.88). The worst result was observed with using of feed from Haltáp company (FCR = 1.37). In this trial, the costs per one kilogram weight gain were relatively balanced. Despite the high price of feed (51 CZK.kg^{-1}), the lowest cost was observed at CatCo feed SELECT-13 EF (42 CZK.kg^{-1}), due to its prosperous feed conversion ratio. Followed by feeds Haltáp (44 CZK.kg^{-1}) and CatCo GROWER-12 EF (45 CZK.kg^{-1}).

In repeatedly tested special feeds for African sharptooth catfish, quite balanced costs per one kilogram of weight gain were achieved. The best tested feed was CatCo SELECT-13 EF (costs per 1 kg of weight gain were 41 and 42 CZK) which was also the most expensive feed. On the contrary, the worst was the cheapest feed Haltáp (costs per 1 kg of weight gain were 45 and 44 CZK). Also, the Haltáp feed had less favourable physical properties, as a considerable amount of dust particles and easy disintegrates in water. If the feed ration is immediately consumed by fish, the large feed disintegrating is not critical. Both of these factors can have an adverse effect on higher content of suspended solids in water. It induces higher need of capacity of mechanical

filters or sedimentation tanks, and increases the risk of impaired function of biological filters. This need for any intensified exchange of supplementary water requires higher operating costs (higher water and energy consumption for its heating). These factors unequivocally favours at African sharptooth catfish rearing in RAS by using of CatCo SELECT-13 EF feed. If this feed will be used under operating conditions in compliance with good feeding technique and less frequent harvesting which disturbs the fish (in comparison to annotated results in the experiments), further potential reduction of feed conversion ratio to 0.7–0.8 and thereby reduction of feeding cost per one kilogram of weight gain to 35–40 CZK can be expected.

4.7. Fillet yield and product quality

4.7.1. Technological procedure

Fillet yield (Fig. 30 and 31) was determined as the weight percentage of fillets without skin and without pectoral fins (Fig. 32). During fish processing, all visceral organs, pectoral fins, the head, as well as skin and spine were removed (Fig. 33). The next part of the experiment was determination of the protein and fat contents in dry matter of fillets provided in the Laboratory of Genetics, Breeding and Nutrion at the Faculty of Agriculture USB (Dipl.-Ing. Jaromír Kadlec, Ph.D.). At the sensory evaluation, ten people assessed the consistency, smell, taste and aftertaste in order to determine whether there are any differences among tested feeds, and if their composition also influences the quality (in terms of existence of possible positive or negative taste characteristics) of African sharptooth catfish meat (Fig. 34 and 35).

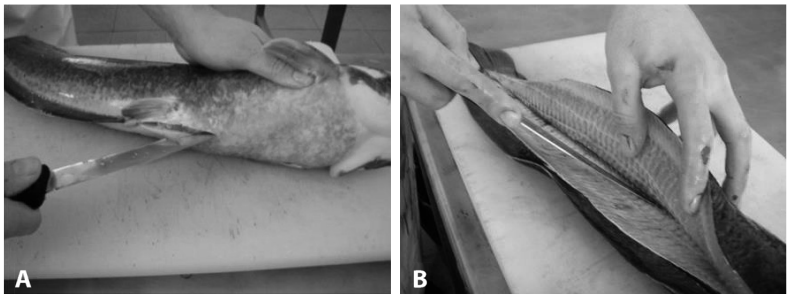

Fig. 30. *Detail gutting (A) and filleting (B) of market size African sharptooth catfish. Photo by J. Kouřil*

Intensive rearing of African sharptooth catfish (*Clarias gariepinus*)

Fig. 31. *Gutting of African sharptooth catfish. Photo by J. Kouřil*

Fig. 32. *Detail of African sharptooth catfish fillets. Photo by J. Kouřil*

Fig. 33. *Skinning (A), the spine with dorsal, tail and anal fins (B), cut part of abdominal area with pelvic fins (C), gonads (D), fillets (E) and head with pelvic fins (F) of African sharptooth catfish. Photo by J. Kouřil*

4.7.2. Results

The basic chemical analysis performed of fillet composition showed about 17% protein and 6% fat contents in dry matter. The highest average values of fillet yield (43%) were detected in CatCo SELECT-13 EF feed. However, the average value of fillet yield slightly exceeded 40% (in all feeds). It did not differ either between the sexes, nor among tested feeds (no statistically significant difference at a significance level of 5%). In terms of quality of the final product, no statistically significant differences were detected in organoleptic muscle (meat) characteristics of African sharptooth catfish fed by various tested feeds (as the tastiest sample CatCo SELECT-13 EF was evaluated by 38% of assessors). It follows that, in terms of product quality, all feeds used in feeding tests are useful and appropriate for African sharptooth catfish.

Intensive rearing of African sharptooth catfish (*Clarias gariepinus*)

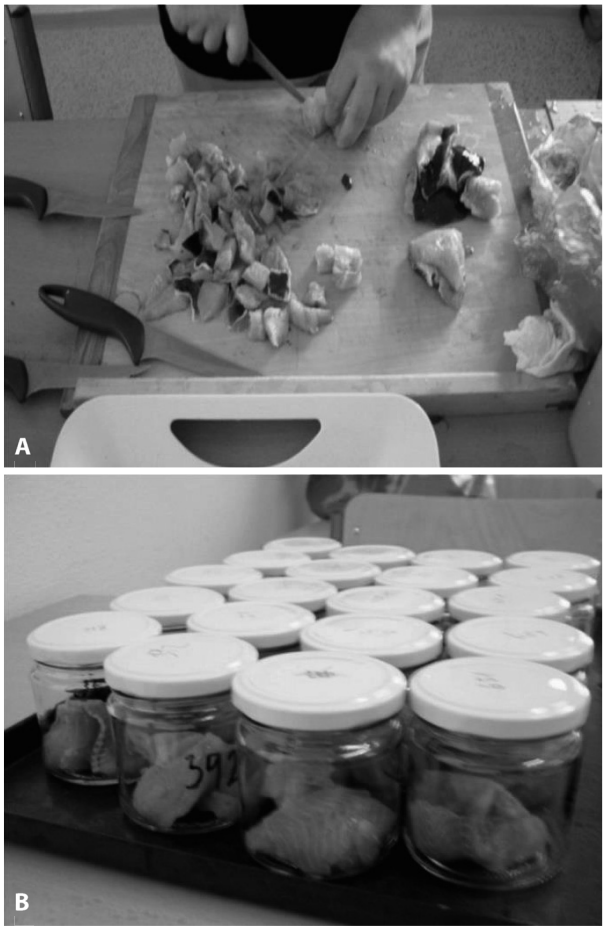

Fig. 34. *Preparation of samples before their heat treatment for organoleptic assessment of African sharptooth catfish muscle. Photo by J. Kouřil*
A: Homogenization of fillets into cubes of approximately 3 x 3 cm;
B: Closed and labelled glass jars with sliced cubes of flesh before cooking.

Fig. 35. *Organoleptic assessment of heat treated samples of African sharptooth catfish muscle by assessors. Photo by T. Zajíc*

Intensive rearing of African sharptooth catfish (*Clarias gariepinus*)

5. ECONOMICAL BENEFIT OF TECHNOLOGY FOR BUSINESS SUBJECT

Technology is designed for specialized farms engaged in fish rearing in recirculation systems with intensive breeding of African sharptooth catfish for efficient production of marketable fish. The purpose of technology is to help solve some problems with rearing of this fish species, in particular, the use of artificial reproduction and optimisation of utilization of commercially produced feeds that will be convenient for African sharptooth catfish.

A user who applies the technology can expect annual gross profit of 400 thousand CZK. This assumption is based on the usual price of marketable fish (80 CZK.kg^{-1}), feed prices (40 CZK.kg^{-1}), feed conversion ratio (FCR = 0.8) and estimated proportion of feed costs in the amount of 50% of total costs. Further costs include the costs for rearing and artificial spawning of broodstock, egg incubation and fry rearing, including starter feeds, energy, water, transport, property depreciation, personnel and insurance costs. In the case of annual production of 25 t of marketable African sharptooth catfish with a total price of 2 million CZK, the annual feed consumption in the amount of 20 t can be expected and thus feed costs reach 800 thousand CZK. The total annual costs of rearing can assume 1600 thousand CZK. The estimated annual gross profit (the difference between revenue from sales of marketable fish and total costs) of farms with annual production of 25 t of marketable African sharptooth catfish should be 400 thousand CZK.

6. APPLICATION OF TECHNOLOGY IN PRODUCTION OF BUSINESS SUBJECT

Technology summarizes the practical experience and the results of a series of partial experiments focused on the problems of broodstock breeding, methods of artificial reproduction, egg incubation and marketable fish production (including effects of commercially produced feeds on fillet yield and meat quality). The aim of technology is to provide, to specialized fish farms, a sophisticated technological method and necessary information for effective intensive farming of African sharptooth catfish for efficient production of marketable fish. This species is very promising for rearing in recirculating aquaculture systems of various sizes using heated water. The main advantages of its rearing can include a short production cycle and high meat quality. African sharptooth catfish breeding allows not only an increase of species diversity of the fish market, but also retention or increase of the production market of Czech aquaculture.

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CHAPTER 5

GENERAL DISCUSSION

Fishes are poikilothermic aquatic organisms and their life processes (development, growth, metabolism, survival, reproduction) are considerably influenced by various external conditions (temperature, oxygen concentration, light, pH, salinity, water currents, feeding availability). Especially, early fish stages (embryos and larvae) are highly sensitive to them, in comparison to juveniles and adults (Kamler, 1992, 2002; Rijnsdorp et al., 2009; Kar, 2015). For this reason, enough information about species-specific optima, viable ranges and limits of external environmental factors are crucial for successful fish rearing. If they are inappropriate, undesirable deviations in fertilization and hatching (Gao et al., 2011), high mortality of early stages (Pepin, 1991), morphological malformations (Dionisio et al., 2012; Paes et al., 2014), inadequate feeding behavior (Rønnestad et al., 2013), failed inflation of swim bladder (Trotter et al., 2003) or cannibalism (Barron et al., 2012) can occur. Therefore, knowledge of effect of environmental conditions on early fish stages are desirable to study for successful production of sufficient amount of quality fish larvae and, subsequently, for rearing juveniles and market size fish for sale.

In the present thesis, early development of African sharptooth catfish was studied in relation to selected exogenous abiotic factors (water temperature and light intensity).

Effect of water temperature (Paper I and III)

The present study investigated the effect of water temperature on the early ontogenetic development of African sharptooth catfish within the thermal range of 17.4–35.6 °C. The embryos and larvae were reared at 33 temperatures from egg fertilization to full yolk absorption during six consecutive experiments with/ without feeding. Early development (incubation and hatching period, timing of the onset of external feeding and yolk absorption, body size, developmental and survival rate) of African sharptooth catfish was influenced by water temperature verigying the observations of Britz and Hecht (1987), Legendre and Teugels (1991), Haylor and Oyegunwa (1993), Adamek (1994), Kamler et al. (1994), Haylor and Mollah (1995), and Hamackova et al. (2007).

Based on the interspecific comparisons of temperature of biological zero and number of effective day-degrees, African sharptooth catfish should be classified as a warmwater species with a short incubation period and a high temperature of biological zero at which ontogeny is theoretically arrested (Kamler, 2002). In the present study (Prokesova et al., 2015), derived effective day-degrees ($D°_{eff}$ = 11.8) and temperature of biological zero (t_0 = 15.4 °C) between the values of $D°_{eff}$ = 9.7 and 13, with t_0 = 17.4 and 14.5 °C, respectively observed by Kamler et al. (1994) and Haylor and Mollah (1995). Similar values of $D°_{eff}$ and t_0 are reported for *Hypophthalmichthys molitrix* ($D°_{eff}$ = 8, t_0 = 16 °C), *Ctenopharyngodon idella* ($D°_{eff}$ = 13, t_0 = 13.6 °C) and *Carassius carassius* ($D°_{eff}$ = 29, t_0 = 14.7 °C), (Kamler, 2002).

The incubation period of African sharptooth catfish was inversely proportional to the water temperature (Legendre and Teugels, 1991; Kamler et al., 1994; Haylor and Mollah, 1995; Prokesova et al., 2015), as for other species (*Cyprinus carpio* – Penaz et al., 1983; *Coregonus albula* – Luczynski and Kirklewska, 1984; *Salmo trutta* – Raciborski, 1987; *Abramis brama* – Kucharczyk et al., 1997). In the present work, incubation period of African sharptooth catfish was 15–70 hPF at 33.6 and 18.9 °C, respectively (Prokesova et al., 2015). The hatching period of African sharptooth catfish was shortened as water temperature increased, 1.4–12.3 h

at 33.7 and 18.9 °C, respectively (Kamler et al., 1994; Haylor and Mollah, 1995; Prokesova et al., 2015). Temperature within the optimal range can be used to manipulate incubation duration, where high temperature can be used to better synchronize hatching (Prokesova et al., 2015), as well in *Chondrostoma nasus* – Kamler et al. (1998), *Eupallasella percnurus* – Kaminski et al. (2006), *Gadus macrocephalus* – Laurel et al. (2008), or *Lepidopsetta polyxystra* – Laurel and Blood (2011).

Endogenous feeding of African sharptooth catfish was reduced with rising water temperature (Viveen et al., 1986; Haylor and Mollah, 1995; Prokesova et al., 2015). Larvae started to intake external feeding after 1.6 – 3.8 dPH (*days post hatching*) at 30.3 and 21.5 °C, respectively. Whereas, the yolk absorption period varied according to the feeding trials (Prokesova et al., 2015). In larvae reared with external food supply, the yolk absorption period was inversely proportional to the water temperature (Viveen et al., 1986; Haylor and Mollah, 1995) and prolongated (as much as six-fold), in comparison with unfed larvae. In summary, the first external feeding should be provided to African sharptooth catfish larvae no later than the second or third day post hatching, because the fishes are capable of ingesting the external food so as to prevent energy deprivation after the yolk is absorbed, which can occur after 2.2–4.6 dPH at the optimal thermal range (Prokesova et al., 2015).

Size of fish at hatching can be influenced by incubation temperature in four different ways. The responses seem to be species-specific as they may vary within one species between various ontogenetic states, as well as between different temperatures (summarized by Kamler, 2008). Firstly, size of newly hatched fish species can be a temperature-independent parameter (*Clupea harengus* – Blaxter and Hempel, 1963; *Misgurnus fossilis* – Drozd, 2011). Secondly, it can correspond to water temperature (*Eopsetta jordani* – Alderdice and Forrester, 1971a; *Gadus morhua* – Pepin et al., 1997). Thirdly, a dome-shaped response of larval size with an ascending limb at lower temperatures and a descending limb at the higher part of the zone of thermal tolerance can occur (*Sardinops caerulea* – Lasker, 1964; *Perca fluviatilis* – Kokurewicz, 1969; *Scomber scombrus* – Mendiola et al., 2007). However, a decrease of larval body size with increasing water temperature is observed in hatched larvae is more common – (*Cyprinodon macularius* – Kinne and Kinne, 1962; *Gadus macrocephalus* – Alderdice and Forrester, 1971b; *Cyprinus carpio* – Penaz et al., 1983; *Coregonus lavaretus* – Escaffre et al., 1995; *Salvelinus alpinus* – Huuskonen et al., 2003; *Salmo trutta* – Ojanguren and Brana, 2003; *Melanogrammus aeglefinus* – Martell et al., 2005; *Lepidopsetta polyxystra* – Laurel and Blood, 2011). The dome-shaped type of size response to increasing temperature was typical also for African sharptooth catfish over the entire tested range. Hatched larvae were smaller (total body length: less than 4 mm) at the lower and higher end (below 22.9 °C and above 30.3 °C, respectively) of tested thermal range (Prokesova et al., 2015). The negative effect of higher temperatures on size of hatched larvae is probably a result of increased embryonic motility, accompanied by earlier formation of hatching gland cells, temperature-stimulated chorionase secretion and higher enzyme activity at increased temperatures. All this may result in earlier of the egg capsule and result in premature hatching of smaller, less developed larvae at higher temperatures (Hayes et al., 1953; Blaxter, 1969 and 1992; Kamler, 2008). Whereas, the smaller size of hatched larvae at the lower end of tested thermal range can be a result of premature hatching caused by a partial loss of fluid from perivitelline space in the cold environment (Alderdice and Forrester, 1974). Other possible mechanisms which might explain these different responses to the water temperature were summarized by Kamler (2008), who pointed out that the interpretation of relationship between body size and incubation temperature can be correct only in the case when the full range of zone of thermal tolerance is assessed, as it was done by Prokesova et al. (2015).

Size of larvae at the first ingestion of external feeding and complete yolk absorption can be a temperature-dependent parameter that is positively or negatively correlated with water temperature (*Pagellus erythrinus* – Klimogianni et al., 2004; *Misgurnus fossilis* – Drozd, 2011), whilst in other fish species (*Chondrostoma nasus* – Kamler et al., 1998; *Melanogrammus aeglefinus* – Martell et al., 2005) it may be a temperature independent parameter that is not influenced by the increasing water temperature (summarized by Kamler, 2008). In African sharptooth catfish, the size of hatched larvae decreased with increasing water temperature (from 7.6 to 7.1 mm at 21.5–30.3 °C) at the onset of mixogenous feeding. However, the size of larvae with full absorbed yolk differed depending on the availability of external food. Size of unfed larvae was not disimilar among the temperatures at full yolk absorption. Whereas, it was inversely proportional to the water temperature (from 16.1 to 12.2 mm at thermal range 27.3–33.2 °C) in fed larvae. It reached, an average of twice the size of the unfed larvae. In summary, from hatching until the full yolk absorption, the larger size fishes were produced during rearing at the lower half of the optimum temperature range (Prokesova et al., 2015).

The zone of thermal tolerance is a range where fish can live without developmental and growth abnormalities (Lapkin et al., 1981). In terms of survival, the zone of thermal tolerance for early development of African sharptooth catfish ranged from 18.9 to 33.7 °C (Prokesova et al., 2015). Within this zone, the temperatures significantly affected the ontogenetic course. Whereas, outside of this range, fishes did not survive (as reported by Brett, 1979 or Kamler, 2002). Lethal temperatures for African sharptooth catfish was below 17.5 and above 35.2 °C during embryonic period, then below 19 and above 33 °C during larval period (Prokesova et al., 2015). Within the zone of thermal tolerance, optimal temperatures, where at least 60% of individuals survive, could be determined (Penaz et al., 1983; Ozernyuk et al., 1987; Kostomarova, 1991). However, survival did not exceed (24–94% and 35–57% for embryonic and larval period, respectively) the recommended value of 60 % during most of the experiments (Prokesova et al., 2015). It may be a result of low egg fertilization (about 40–50%) in African sharptooth catfish (Hamackova et al., 2007), or by the quality of broodstock gametes (Bobe and Labbe, 2010). In spite of this, thermal range between 22.9 and 30.3 °C can be considered as optimal temperatures for African sharptooth catfish early development. This finding is very important for rearing of African sharptooth catfish early stages (Prokesova et al., 2015). It confirmed the existing knowledge about the optimal thermal range for early rearing of African sharptooth catfish (20–35 °C, Haylor and Mollah, 1995; 25–27 °C, Hamackova et al., 2007) and contributed defining the range more precisely.

Effect of light intensity (Paper II)

Light is important abiotic factor that may influence fish from their early life to adult stages in various ways. Yet, the role of light has often been overlooked in comparison to other external factors (Mangor-Jensen and Waiwood, 1995; Boeuf and Le Bail, 1999; Downing and Litvak, 2002; Kamler, 2002). Recently, the effect of photoperiod on African sharptooth catfish early stages and juveniles has been well studied (Britz and Pienaar, 1992; Appelbaum and Kamler, 2000; Mino et al., 2008). However, there is still a dearth of information about the influence of light intensity and wavelength on its development and rearing.

In the present study (Prokesova et al., 2016), the effect of light intensity on the early development of African sharptooth catfish was investigated. Fish were incubated at five light intensities (<0.1, 70, 500, 2500, 8000 Lx) under stable conditions (about 27.2 °C and photoperiod of 24L:0D) with/ without feeding supply. Two experiments were carried out

from egg fertilization until full yolk absorption in African sharptooth catfish. Light intensity influenced survival and development, yolk utilization efficiency and body size of African sharptooth catfish early stages.

Generally, light can have a variable effect on early development of fishes but is more related to the species and age, to maternal effect or environmental adaptations (Boeuf and Le Bail, 1999; Appelbaum and Kamler, 2000; Kamler, 2002; Politis et al., 2014). For instance, positive effect of lightening was observed in *Acipenser stellatus* early stages (Detlaf et al., 1981). On the contrary, negative impact of lightening was recorded in *Engraulis encrasicolus maeoticus*, *Oncorhynchus nerka*, *Ophiodon elongatus* yolk larvae (Leshchinskaya, 1954; Leitritz and Lewis, 1976; Appelbaum et al., 1995). Whereas, no influence of light was found in *Rutilus rutilus heckeli* or *Stizostedion lucioperca* larvae (Belyj, 1961).

Here, the incubation period of African sharptooth catfish ranged from 24.3 to 29.9 h at 27.2 °C under the brightest light intensity and darkness, respectively (Prokesova et al., 2016). These values are comparable to those of other authors for this species at similar temperatures (22–33 h at 25–28 °C; Legendre and Teugels, 1991; Adamek, 1994; Haylor and Mollah, 1995). Generally, much shorter incubation periods have been observed also during studying of African sharptooth catfish with smaller eggs (17 h in 1 mm eggs in Olaniyi and Omitogun, 2013, compared to larger eggs of about 1.5 mm in diameter Prokesova et al., 2016).

The relationship between duration of egg hatching was curvilinear. Time to hatching of 50% of individuals was about 25% longer (6 h) under darkness than under the brightest light intensity. This finding confirmed the results of Mino et al. (2008) that eggs of African sharptooth catfish have the shortest hatching time under photoperiod of continuous light, but they also can be sucessfully hatched under different photoperiods. Earlier hatching under bright light has been observed also in other fish species (*Paralichthys dentatus*, Watanabe et al., 1998; *Hippoglossus hippoglossus*, Downing and Litvak, 2002; *Clarias macrocephalus*, Mino et al., 2008). The phenomenon of earlier hatching under bright light may be partly caused by increased muscular movement frequency resulting in a more precocious rupture of the chorionic membrane. The associated hatching of African sharptooth catfish at an earlier developmental stage under more intense lightening was largely supported by the negative correlation between the light intensity and the body length of hatchlings (Prokesova et al., 2016). After Boeuf and Le Bail (1999), light may stimulate increased production of retinal cones that are supposed to be very important for further embryonic development and growth. According to the literature, the relatively earlier hatching of eggs exposed to continuous lightening was probably due to accelerated development via the light-retina-retinoic acid that signalizes organogenesis pathway (Ralph, 1975; Reiter, 1986; Arendt, 1995; Boeuf and Le Bail, 1999; Gilbert, 2000; Mino et al., 2008).

In synthesis, African sharptooth catfish is a tactile-chemoreceptive predator that relies little on vision (Hecht and Appelbaum 1987, 1988). Shortly after hatching, the larvae initiate their photophobic behaviour (Hogendoorn et al., 1980; Hecht and Appelbaum, 1988; Appelbaum and Kamler, 2000). In the present study, African sharptooth catfish started to ingest the external feeding (decapsulated cysts of *Artemia salina* L.) on the fourth day post fertilization at all light intensities. While, Appelbaum and Kamler (2000) observed a delay of the onset of mixogenous feeding by five hours under light conditions compared to darkness. At the same moment, the remaining yolk was larger in larvae under the most intense lightening, whereas

the individual wet weight did not differ among the light treatments. Nevertheless, the size of larvae still varied with light intensities. Fish reared under the brightest light intensity were slightly smaller than the others (Prokesova et al., 2016). Similar trends were observed also in other studies on the same species (Britz and Pienaar, 1992; Appelbaum and McGeer, 1998; Appelbaum and Kamler, 2000; Mino et al., 2008). The slower growth accompanied by higher growth heterogeneity and subsequent cannibalism in African sharptooth catfish larvae and juveniles is generally interpreted as the outcome of enhanced aggression, resulting in lower food ingestion under bright light than under dim conditions or permanent darkness (Hecht and Appelbaum, 1988; Britz and Pienaar, 1992; Appelbaum and McGeer, 1998; Appelbaum and Kamler, 2000; Almazan-Rueda, 2004; Mino et al., 2008). Besides, better growth of African sharptooth catfish under dim conditions could be caused by minimizing the energy utilization for locomotor activity, whilst the energy investment in growth was maximized (Appelbaum and Kamler, 2000). According to Reiter (1986), total darkness might also induce a production of melatonin (a weight regulatory hormone) which is usually synthesized and secreted by the pineal gland during the dark phase of photoperiod.

Here, complete yolk absorption occurred 11 days post fertilization in externally fed larvae. Both body length and weight decreased with increasing light intensity. While, no effect of light intensity was found on both parameters in fish reared without external feeding. Their body weight was about 15–20% lower than in larvae reared with feeding supply. However, it should be noted that the growth was very slow. The fastest growing fish (reared under darkness) did not double their weight after eight feeding days (Prokesova et al., 2016). Probably, the slow growth was caused by improper decapsulation of *Artemia* cysts resulting in very poor food absorption; many intact cysts were observed exiting the intestine of photographed larvae. This somehow compromises the relevance of present observations relative to recommendations for hatchery managers. On the other hand, the results probably indicate that the light-dependent growth patterns observed in situations of rapid growth also apply to situations of slow growth. This may indirectly suggest that differences in aggressiveness might suffice to produce such patterns, as the motivation to feed was supposedly high under all light intensities in contexts of very slow growth and low food absorption rates (Prokesova et al., 2016). In accordance with previous statement, Mino et al. (2008) confirmed that the larval growth can be significantly affected by light in conjuction with the type of a diet. Besides, the efficiency of detecting and catching the food particles by sight can be improved by different color contrasts of tank background during rearing of African sharptooth catfish (Lee et al., 2014). In comparison to other studies (Appelbaum and McGeer, 1998; Almazan-Rueda et al., 2005), Mino et al. (2008) summarized that optimal growth differs also with life stages in African sharptooth catfish with larvae generally growing best under continuous darkness, while juveniles need shorter periods of light for better growth.

Studies on the eggs of other fish species have demonstrated contrasting trends, sometimes with mortality being negatively correlated with light intensity (*Rhombosolea tapirina* – Hart et al., 1996), while in other cases, egg mortality was higher under low light whereas the proportion of deformed hatchlings with increasing light intensity (*Hippoglossus hippoglossus* – Bolla and Holmefjord, 1988; *Epinephelus striatus* – Ellis et al., 1997). In the present study, the mortality of African sharptooth catfish during the egg incubation period was proportional to light intensity (Prokesova et al., 2016). A possible explanation of this response may be elevated sensitivity to ultraviolet radiation during very early fish ontogeny, where the differentiation rate is at the highest (Cai, 1993; Strähle and Jesuthasan, 1993). Besides, concentration of a substance acting as a sun screen is increased with age of fish

(Hofer and Kaweewat, 1998). Similarly, Mino et al. (2008) observed the highest hatching rate of African sharptooth catfish, as well as *Clarias macrocephalus*, under longer dark photoperiods. In African sharptooth catfish, daily mortality during egg incubation was much higher (>50 % day^{-1}) than in yolk larvae feeding endogenously (7.5% day^{-1}) or mixogenously (6.7 % day^{-1}). It is possible that the high mortality during the embryonic period partly reflected a rather low fertilization rate, as suggested by the observation of numerous decaying eggs no later than 8 hours after fertilization (Prokesova et al., 2016). Appelbaum and Kamler (2000) observed the inhibition of African sharptooth catfish survival rate by lightening during the endogenous feeding. Conversely, Britz and Pienaar (1992) did not record any differences in its survival rates. Nevertheless, here the best survival rates were observed under intermediate light intensities (500 or 2500 Lx), and the lowest values under the brightest lightening (8000 Lx) or dim conditions (<0.1 or 70 Lx) after hatching (Prokesova et al., 2016).

CONCLUSION

In summary, we have demonstrated clearly that African sharptooth catfish early stages have high thermal requirements typical for warmwater fish species with a high temperature of biological zero (t_0 = 15.4 °C). The early development, growth and survival are significantly influenced within the zone of thermal tolerance (18.9–33.7 °C). Whereas, outside of this range, the temperatures are lethal. The optimal temperature range of 22.9–30.3 °C is recommended for higher survival of African sharptooth catfish during early rearing until the yolk is fully absorbed. For better synchronization of hatching and shortening of the incubation period, the upper temperature can be used. In terms of light, the present study provided evidence about the detrimental effect of continuous bright light (8000 Lx) on African sharptooth catfish early stages, since it negatively affected growth and survival rate of larvae. Therefore, the eggs should be incubated in darkness, followed by rearing larvae under dim light or intermediate light intensities (70–500 Lx), as these can provide the best survival rates and facilitate the work of hatchery operators while minimizing electricity costs. Nevertheless, several questions regarding the light still remain to be answered. Are these light intensities optimal also for rearing juveniles and adults of African sharptooth catfish? What effect has interaction between light intensity and stocking density? Or, which light wavelengths are optimal for rearing of African sharptooth catfish?

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ENGLISH SUMMARY

Effect of temperature and light intensity on early development of African sharptooth catfish in commercial production

Markéta Prokešová

The successful production of quality early fish stages is crucial for artificial rearing of fishes. Nevertheless, it is quite a problematic process, because fish as poikilothermic organisms are highly sensitive to various environmental conditions. Moreover, both fish embryos and larvae are more affected by biotic and abiotic factors (such as predation, stocking density, temperature, light, pH, salinity, oxygen saturation, feeding availability, or their interactions) than older juveniles or adults. Most fish processes such as reproduction, metabolism, survival, development and growth can be influenced by these conditions. Therefore, it is important to understand their impacts on fish stages during embryonic, larval, juvenile, adult, and eventually the senescent period. Then, following the application of new knowledge to the practise, especially species- and age-specific optimal conditions, should result in higher survival, better growth rate, well developed fish without deformities and lower cannibalism.

The objectives of the present thesis were to assess the effect of water temperature and light intensity on the early development (embryonic and larval period) of African sharptooth catfish as a prospective fish species for freshwater intensive aquaculture. Due to its positive characteristics (for instance fast growth, ability to breathe atmospheric air, rearing at high stocking density) this species can meaningfully contribute to the global aquaculture production which is expected to rise in the coming years. However, there are still some difficulties in the early rearing African sharptooth catfish regarding the cannibalism, feeding, survival and necessity of detailed information about the effects of some abiotic factors and their interactions.

The results of the study provide information on the influence of water temperature during early development on growth and survival from egg fertilization until the end of yolk absorption. This fish can be classified as a warmwater species, characterized by a short incubation and hatching period that are inversely proportional to the water temperature (PF = post fertilization; 15–70 hPF at 33.6 and 18.9 °C; 1.4–12.3 h at 33.7 and 18.9 °C). The endogenous feeding period was reduced by rising water temperatures; they start to take external food on 1.6–3.8 dPH at 30.3 and 21.5 °C, respectively. Also, yolk absorption was affected in the same way by the water temperature (PH = post hatching; 7.4–15.8 dPH gradually at 33.2 and 27.1 °C). The yolk volume in hatched larvae positively correlated with the size of spawned eggs, however it was negatively associated with increasing temperature at the first external food ingestion. The size of larvae reared with food supplied was inversely proportional to the water temperature within the optimum thermal range at hatching (up to 5 mm), ingestion of the first food particles (up to 7.6 mm), as well at the full yolk absorption (up to 16.1 mm). In terms of survival, the optimum temperatures ranged between 22.9–30.3 °C within the zone of thermal tolerance (18.9–33.7 °C) denoting the temperatures allowing survival of its early stages. The temperature of biological zero that theoretically arrested the early development of African sharptooth catfish was determined as 15.4 °C.

Based on the study investigating the effects of light intensity on the early development, the growth and survival of African sharptooth catfish from egg fertilization until the full yolk

absorption, the highest light intensity (8000 Lx) was concluded as truly detrimental to its early stages. The incubation period was shortened (about 25%) with increased lightening from 24.3 to 29.9 h at an incubation temperature of 27.2 °C under the most intense light intensity and darkness, respectively. Fish were smaller at hatching at precocious developmental stages under the brightest light compared to lower light conditions, and, they started to ingest external food on the fourth day post fertilization at all light intensities. Nevertheless, their size still varied among the different lightening. The larvae were slightly smaller with larger remaining yolk under the highest intensity than under the others. Complete yolk absorption occurred after 11 days post fertilization in externally fed larvae. At that time, both their body length and weight decreased with increasing light intensity. The mortality during egg incubation was proportional to the light intensity, for reasons that remain to be clarified. The daily mortality was much higher in egg incubation (>50% day^{-1}) than in yolked larvae feeding endogenously (7.5% day^{-1}) or mixogenously (6.7% day^{-1}). Nevertheless, the lowest mortality rates were observed under intermediate light levels (500 or 2500 Lx), and the highest values under the brightest lightening (8000 Lx) or dim conditions (<0.1 or 70 Lx) after hatching. If a single light intensity is to be chosen for all early stages until the end of yolk absorption, the preferred intensity should be 70–500 Lx rather than darkness or bright light.

In summary, African sharptooth catfish eggs should be incubated and larvae reared within the optimum temperature range between 22.9–30.3 °C. Also, eggs should be incubated in darkness, with subsequent larval rearing under dim light or intermediate light intensities (70–500 Lx), so as to facilitate the work at hatcheries.

CZECH SUMMARY

Vliv teploty a intenzity světla na raný vývoj sumečka afrického v komerčním chovu

Markéta Prokešová

Úspěšná produkce dostatečného množství kvalitních larev je klíčová pro umělý odchov všech druhů ryb. Tento proces je však u ryb, jako studenokrevných živočichů citlivých vůči podmínkám prostředí, poněkud problematický. Zejména jejich raná stadia (embrya a larvy) jsou více ovlivňována různými biotickými a abiotickými faktory (např. predace, hustota obsádky, teplota, světlo, pH, salinita, kyslík, dostupnost potravy a interakce těchto faktorů). Většina životních procesů ryb (reprodukce, metabolizmus, přežívání, vývoj a růst) může být významně (pozitivně, či negativně) ovlivňována těmito faktory. Porozumění jejich vlivům na embryonální, larvální, juvenilní, adultní a případně senescentní periodu ryb je proto velmi žádoucí. Aplikace nových znalostí do praxe (zejména o druhově a věkově specifických optimálních podmínkách) by totiž mohla vést k vyššímu přežívání ryb, lepšímu růstu, správnému vývoji, snížení výskytu malformací či k nižší frekvenci kanibalizmu.

Cílem této dizertační práce bylo posoudit vliv teploty vody a intenzity světla na raný vývoj (embryonální a larvální periodu) sumečka afrického, který je perspektivním druhem pro sladkovodní intenzivní akvakulturu. Díky skvělým vlastnostem (např. rychlý růst, schopnost dýchat atmosférický kyslík, chov při vysokých hustotách obsádky) by se tento druh mohl významně podílet na globální produkci akvakultury, jejíž růst je očekáván v následujících letech. V raném odchovu sumečka afrického je však stále několik obtížností týkajících se kanibalizmu, krmení, přežívání a nedostatku informací o vlivech některých biotických a abiotických faktorů a jejich interakcí.

Výsledky studie poskytly informace o vlivu teploty vody na raný vývoj, růst a přežívání sumečka afrického od oplození jiker až po konec absorpce žloutkového váčku. Bylo potvrzeno, že tento druh lze zařadit mezi teplomilné ryby charakterizované krátkou inkubační periodou i dobou líhnutí. Oba intervaly se zkracovaly se zvyšující se teplotou vody (15–70 hodin po oplození při 33,6, resp. 18,9 °C; 1,4–12,3 hodin při 33,7, resp. 18,9 °C). Také perioda endogenní výživy byla redukována s rostoucí teplotou vody. Ryby začaly přijímat vnější potravu 1,6–3,8 dne po vylíhnutí při 30,3, resp. 21,5 °C. Perioda absorpce žloutkového váčku byla teplotou ovlivněna stejným způsobem (7,4–15,8 dnů po vylíhnutí při 33,2, resp. 27,1 °C). Objem žloutkového váčku vylíhlých larev odpovídal velikosti vytřených jiker. Při prvním příjmu vnější potravy byl ale negativně asociován se vzrůstající teplotou vody. Velikost krmených larev se snižovala s rostoucí teplotou vody v rozmezí optimálních teplot při líhnutí (až 5 mm), prvním příjmu vnější potravy (až na 7,6 mm), stejně jako při úplné resorpci žloutkového váčku (až 16,1 mm). Z hlediska přežívání se zóna teplotní tolerance, označující teploty umožňující přežívání raných stadií sumečka afrického, pohybovala mezi 18,9–33,7 °C. Uvnitř této zóny se pak nacházelo teplotní optimum 22,9–30,3 °C vhodné pro odchov embryí a larev tohoto druhu. Biologická nula, která udává minimální teplotu pro zahájení raného vývoje sumečka afrického, byla stanovena na 15,4 °C.

Studie zabývající se vlivem intenzity světla na raný vývoj, růst a přežívání sumečka afrického od oplození jiker po úplnou absorpci žloutkového váčku, ukázala, že nejvyšší zvolená intenzita světla (8000 Lx) byla vyhodnocena jako škodlivá pro raná stadia tohoto druhu. Inkubační perioda při nejvyšší intenzitě světla byla zkrácena přibližně o 25 % (od 24,3 do 29,9 h při

inkubační teplotě 27,2 °C) v porovnání s úplnou tmou. Při nejvyšší intenzitě se líhnuly nejmenší ryby na nižším ontogenetickém stupni, naopak při úplné tmě se se líhnuly největší jedinci ve vyšším vývojovém stupni. Během čtvrtého dne po oplození larvy začaly přijímat vnější potravu ve všech intenzitách světla. Jejich velikost se lišila podle intenzity osvětlení. Nejmenší larvy s největším žloutkovým váčkem byly pozorovány pod nejvyšší intenzitou světla. Jedenáctý den po oplození měly krmené ryby resorbovaný žloutkový váček ve všech intenzitách světla. Délka jejich těla byla závislá na intenzitě osvětlení, klesala se zvyšující se intenzitou světla. Během inkubační periody se mortalita zvyšovala se zvyšující se intenzitou světla. Denní mortalita byla mnohem vyšší při inkubaci jiker (>50 % x den^{-1}) než u larev při endogenní (7,5 % x den^{-1}), nebo mixogenní výživě (6,7 % x den^{-1}). Po vylíhnutí byla však nejnižší mortalita pozorována pod středními intenzitami (500 a 2500 Lx), naopak nejvyšší hodnoty mortality byly pozorovány pod nejintenzivnějším (8000 Lx) a nízkým osvětlením (<0,1 a 70 Lx). Při možnosti výběru jediné intenzity světla pro inkubaci jiker a raný odchov larev do stadia úplné resorpce žloutkového váčku by byla intenzita v rozmezí 70–500 Lx vhodnější než úplná tma či intenzivní světlo.

Na základě výsledků této dizertační práce lze doporučit inkubaci jiker a odchov larev sumečka afrického v rozmezí optimálních teplot 22,9–30,3 °C. Inkubace jiker by měla probíhat ve tmě a následný odchov larev pak při tlumeném světle nebo středních intenzitách světla (70–500 Lx), které jsou vhodné i pro práci v líhních.

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Prokesova, M., Żarski, D., Stejskal, V., Czerniak, E., Blecha, M., Palińska-Żarska, K., Krejszeff, S., Drozd, B., Gomulka, P., Laczynska, B., 2014. Effect of various light conditions on food intake, growth and cannibalism of Eurasian perch, *Perca fluviatilis* (Linnaeus, 1758) juveniles. In: Aquaculture Europe 2014, October 14–17, Donostia San Sebastian, Spain *(Poster presentation)*.	2014
Prokesova, M., Stejskal, V., Matousek, J., Kouril, J., 2015. Effect of various light intensities on early ontogeny of African catfish *Clarias gariepinus* (Burchell, 1822). In: Aquaculture Europe 2015, October 20–23, 2015, Rotterdam, Netherlands *(Oral presentation)*.	2015

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