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Morphological changes during the transition from freshwater to sea water in an amphidromous goby, *Sicyopterus lagocephalus* (Pallas 1770) (Teleostei)

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Abstract – The widely distributed amphidromous goby *Sicyopterus lagocephalus* needs drastic change of habitat to fulfil its life cycle: adults live and spawn in rivers, where eggs hatch into prolarvae that have to reach the sea to acquire characteristics of planktonic larvae. Postlarvae return to rivers where they recruit and grow to the adult reproductive stage. Here, we describe the prolarval stages, namely from hatching to first contact with sea water, as well as the first marine larval stages. The observations were made under experimental conditions. We described 3 prolarval substages in freshwater (L1a–L1c). Prolarvae present a slight but visible ontogenetic development in freshwater, during which the yolk sac begins to reduce, the pigmentation increases on the body and in the eyes, and the lenses appear, although the eyes are not functional. Prolarvae need to reach the sea in a maximum of 96 h to pursue their development. Their transfer in sea water at a salinity of 36.5 induces important morphological modifications (i.e. yolk sac full absorption, appearance of pectoral fins, migration of the eyes in anterolateral position of the head, opening of mouth and anus), enabling the organisms to adapt to their new environment. This marine stage is divided into two substages: L2a corresponding to the organisation of the morphological structures adapted to the marine environment and L2b during which these morphological structures become functional. Whether it is in freshwater or sea water, the duration of the substages depends on the water temperature, but is similar for all individuals for a given temperature.

Key words: larval development; larval morphology; amphidromy; *Sicyopterus lagocephalus*; Reunion Island

Introduction

Migration strategy occurs in some species in response to heterogeneity of environmental conditions (Dingle 1996). The transfer of an individual or a population to places where their survival is facilitated (e.g. more important or more accessible food resource) or their reproduction is more favourable (e.g. absence of predator for the offspring) increases the fitness of the individuals, on condition that the benefit for the species exceeds the migration costs (Hinch et al. 2006; Metcalfe et al. 2002). Migration also favours the colonisation of distant habitats, and thus a wider distribution of the species.

Such migratory strategies are commonly observed for fish populations.

However, migration is associated to a strong mortality. There also is a risk that the organisms do not find a suitable habitat in time to fulfil their life cycle (Jonsson & Jonsson 1993).

Diadromy is a particular kind of migration in which species shift between two radically different

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environments, namely freshwater and sea water, during their life cycle. Amphidromy, as defined by McDowall (1988, 1992), is a type of diadromy in which the migration between these two systems is not related to the reproductive event.

In tropical insular rivers, the diversity of fish communities and then biodiversity rely mainly on amphidromous gobies, at least for the Caribbean and Indo-Pacific islands, and most of these species are endemic (Lim et al. 2002; Keith 2003; Marquet et al. 2003; Keith et al. 2005; Lord & Keith 2008; Keith & Lord 2011). Indeed, the amphidromous life cycle is particularly adapted to these environments as it allows colonisation or recolonisation of new habitats, through its dispersive stage. Tropical insular rivers constitute highly unstable systems, because of the extreme climatic and hydrological variations they undergo on a seasonal scale (Keith 2003; Keith et al. 2010). In this context, the rivers are frequently subject to local extinctions of their fauna that are compensated by recolonisation through the larval dispersive stage of amphidromous species (McDowall 2008).

Sicyopterus lagocephalus (Pallas 1770) (Teleostei: Gobiidae: Sicydiinae) belongs to the Sicydiinae subfamily, regrouping 110 species and nine genera. All of them are amphidromous and are distributed in tropical or subtropical areas (Keith & Lord 2011). *Sicyopterus lagocephalus* is an amphidromous goby, characterised by adults living and spawning in freshwater. After hatching, the prolarvae (or free embryos) drift downstream to the sea, where they transform into more developed larvae which undergo an oceanic dispersive phase. Then, postlarvae return to the rivers where they metamorphose into juveniles (Keith et al. 2008; Taillebois et al. 2011) and grow to the reproductive adult stage. Thus, *S. lagocephalus*, like all amphidromous species, has to shift twice between freshwater and sea water and that implies great anatomical, physiological and behavioural adaptations (Valade et al. 2009; Iida et al. 2010; Ellien et al. 2011).

Besides their importance regarding their strong contribution to river fauna biodiversity, one of the main interests of Sicydiinae species consists in their high economic value. At certain time of the year, some amphidromous gobies are targeted by traditional intensive fisheries, at their postlarval stage, when they migrate back to rivers. They constitute an important source of food for local populations. This is the case in particular in Reunion Island (Mascarene Archipelago, Indian Ocean), where *S. lagocephalus* is subject to strong although unsustainable harvest. Indeed, on account of the complexity of the species life cycle associated to the instability of the river systems, *S. lagocephalus* juvenile stock and thus reproductive adult stock are highly fragile and show some signs of decline.

In this context, the biology and ecology of *S. lagocephalus* need to be better understood, to elaborate management and conservation plans, aiming at preserving both stocks and biodiversity.

In Reunion Island, the freshwater stages of *S. lagocephalus* have been studied, especially its postlarval (i.e. 'bichique'), juvenile and adult stages (Keith et al. 2006, 2008). The prolarval stage, which also takes place in freshwater, and the first larval marine stages have been described too (Valade et al. 2009). However, this latter description was incomplete on some aspects. From then on, a rigorous and accurate description of the morphological modifications, characterising the transformation of the prolarvae into larvae during their passage from freshwater to sea water, is required and constitutes the purpose of this study. It is indeed important to know precisely each step of the larval cycle and how it modifies the morphology and physiology of the animals. This knowledge will also be useful for endocrinological studies, to be able to assess the impact of different hormones on the prolarvae transformation as soon as it begins. Moreover, we aim at establishing a temporal reference table of this transformation chronology that would allow determining precisely at which stage of larval development is situated a larva at the time of its observation.

Material and methods

Review on biological material

In Reunion Island, *S. lagocephalus* freshwater stages have been studied for two decades, so that it is well known that adults are rheophilic and live in clear and well-oxygenated waters (Keith 2003; Keith et al. 2006; Teichert et al. 2014).

Reproduction occurs in rivers, continuously during the year, although it is more intense between January and June, thus coinciding with summer and autumn in the Southern Hemisphere (Delacroix & Champeau 1992; Hoareau et al. 2007; Teichert et al. 2013).

Eggs are laid as clusters on the undersides of rocks and pebbles. Each clutch counts between 5000 and 120,000 small eggs (Delacroix & Champeau 1992), with an average number of around 30,000 eggs (Teichert et al. 2013). The male takes care of the clutch (Keith 2003) until the eggs hatch into prolarvae $\pm$48 h after fertilisation (Delacroix & Champeau 1992).

Temperature plays a major role on the survival and development rate of fish larvae. In Reunion Island, Valade et al. (2009) empirically show that prolarvae survival is optimised for temperatures between 20

and 23 °C, which correspond to mean daily river temperatures during the main spawning period of *S. lagocephalus* (i.e. wet season in this tropical region). This austral season is associated with strong rainfalls that induce a more turbulent river flow and then both a better oxygenation of the water and faster currents. A better water oxygenation facilitates the survival of the eggs and increases prolarval health. As for the fast currents, they allow a rapid transport of the prolarvae to the sea.

During this transport, prolarvae are not totally passive, even if they are unable to resist the current. They oscillate between the bottom and the surface of the water, by swimming up and sinking down alternatively. This vertical behaviour has been observed both in the field (Keith 2003) and in experimental tanks (Ellien et al. 2011), as well as on *Sicyopterus japonicus* prolarvae (Iida et al. 2010). This behaviour is supposed to accelerate the transport of the prolarvae to the sea by increasing the time they spend at the surface where the current flows faster.

In Reunion Island, according to the river length (36 km for the longest) and their flow intensity, prolarvae tumble down the river in <24 h whatever the altitudinal location of the clutches. Experimentally, Ellien et al. (2011) assessed that prolarval survival in freshwater do not exceed 96 h.

Once in the sea, prolarvae undergo the modifications that turn them into planktonic marine larvae. This transition induces food intake, first endogenous with the consumption of yolk sac, and then exogenous when the mouth and anus are open (Ellien et al. 2011). At sea, larvae are planktonic and planktotrophic, with their mouth open in terminal position (Valade et al. 2009; Ellien et al. 2011). In a physiological point of view, larval osmoregulation needs to evolve, to enable the organisms born in freshwater to survive in sea water. This transfer from freshwater to sea water is critical and generates a strong mortality, in experimental conditions (Ellien et al. 2011).

The duration of the marine larval stage of *S. lagocephalus* has been estimated by otolith microstructural analysis and lasts 4–9 months (i.e. exactly between 133 and 266 days) (Hoareau et al. 2007; Lord et al. 2010). Such a long dispersive period, along with an important variability in the pelagic larval duration (PLD) at the annual scale, allows the colonisation of distant habitats and could explain the wide distribution of the species at the Indo-Pacific scale (Keith et al. 2005; Lord et al. 2012).

At the end of the oceanic dispersive stage, postlarvae gather in large numbers at the mouths of the rivers, as they need to find river systems to fulfil their life cycle (Keith 2003). Settlement in freshwaters occurs by successive waves, function of the lunar cycle and hydrological conditions. A second transformation takes place there, under the control of thyroid hormones, allowing them to adapt again to a freshwater environment, after several months spent at sea. During this metamorphosis, their mouth migrates in subinferior position, and the pelvic fins merge to form a sucker, making the juveniles fit to a benthic herbivorous way of life and enabling them to migrate upstream (Taillebois et al. 2011) to settle and reproduce once they have reached the adult stage.

Valade et al. (2009) described five marine larval stages, observed for *S. lagocephalus*, without feeding and for a water temperature between 20 and 23 °C. The L1 stage comprises four substages that Valade et al. (2009) defined as free embryos: L1a stage defined as larvae at hatching and being the unique freshwater stage of this larval development chronology. It is characterised by no trace of eye, no sign of jaw, translucent and yolk sac at its maximum size. The following stage, L1b stage, is defined as larvae just arriving at sea, with 0–20 h spent in sea water and is characterised by early eyes (lens present, retina not pigmented), eyes on lateral sides of the head, posterior tip of mandible detectable as a prominence below the eye, body not pigmented and beginning of yolk sac absorption. The L1c stage is defined as larvae with 20–40 h spent in sea water and is characterised by lens present and pigment beginning to appear on retina, beginning of the migration of the eyes in anterolateral position of the head, mouth is formed but closed, appearance of pectoral fins and multiplication of chromatophores on the body. The L1d stage is defined as larvae with 40–65 h spent in sea water and is characterised by pigmented lens, eyes in anterolateral position of the head, mouth open in subinferior position, developed pectoral fins and appearance of chromatophores in the cephalic area. The last stage corresponds to the L2 stage, with larvae spending more than 65 h in sea water and is characterised by an open mouth in terminal position and operating, the ability to intake and digest exogenous food, no more yolk sac, many chromatophores in the cephalic area and along the body and all internal organs in place.

Sampling protocol

For this study, we used data collected during three field surveys: in April 2010, between April and May 2012 and between April and June 2013. In all cases, *S. lagocephalus* egg clusters were collected in the lower reaches of the Langevin River (south-west of Reunion Island, Mascarene Archipelago, Indian Ocean), at three stations located between 600 m and 2 km from the river mouth (Fig. 1). Alternating between three sampling stations reduces the impact

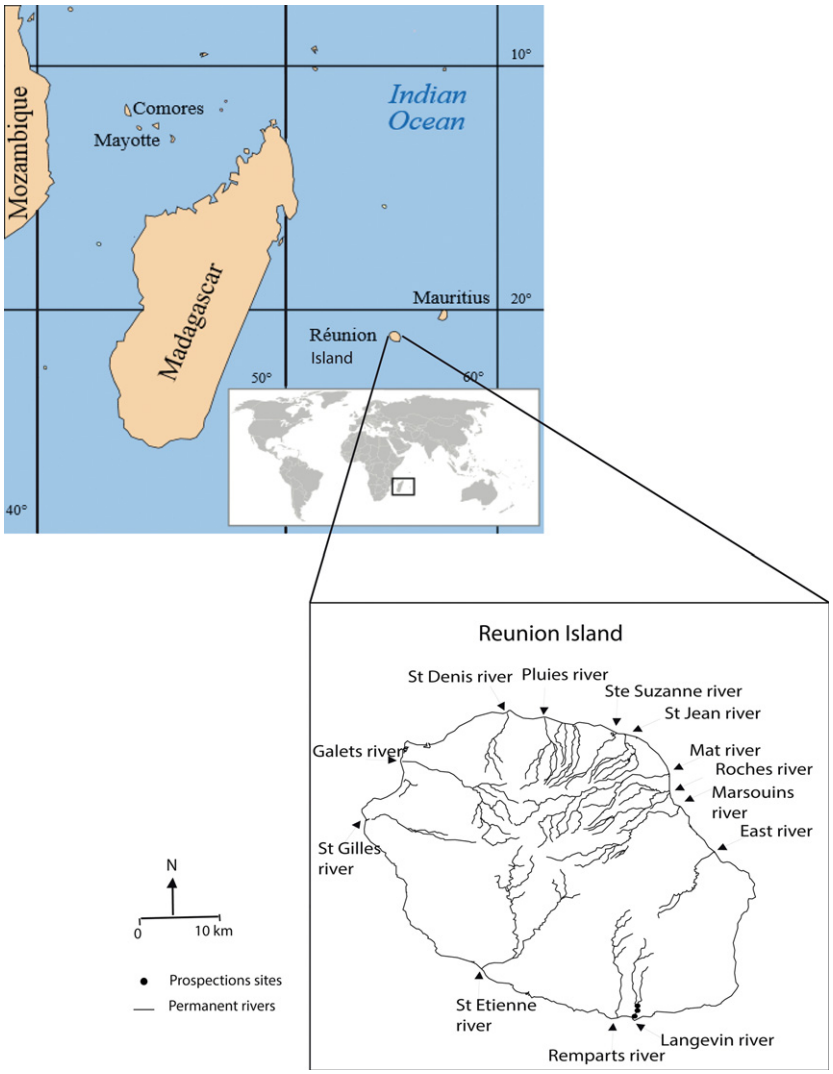

Fig. 1. Location of Reunion Island in the south-west Indian Ocean (left). Location of the rivers in Reunion Island (right). Black dots in Langevin River indicate sampling sites.

of the collect on *S. lagocephalus* population, by enabling a turnover of the clutches at each station.

This river is of torrential type, characterised by an average flow of 1.18 $m^3 \cdot s^{-1}$ (Teichert et al. 2014) and an average temperature between 20 and 23 °C (Valade et al. 2009).

The egg clutches are stuck on the inferior side of the rocks. The rocks that are liable to shelter a clutch show some characteristics: they are heavy (more than 1 kg) and embedded in sand and gravels on the bottom of the river, so that they are quite stable in the context of the strong river flow.

To collect the egg clutches, we had to turn the rocks over one after the other to find them. The newly laid eggs are white and turn grey when they are ready to hatch; we therefore collected only grey clutches. At each sampling, 3–4 grey clutches were collected. Even if one egg cluster produces enough prolarvae to ensure the observations of the different steps of their transformation once in sea water, mixing several clutches reduces the risk of failure (e.g. larvae malformation) linked to a genetic problem on one particular clutch. This study aims at describing the prolarval and larval stages and substages, during their transition from freshwater to sea water, and so, a qualitative approach was judged sufficient. Moreover, larval density at the time of their transfer from freshwater to sea water does not influence larval development (i.e. the same developmental pattern, the same chronology and the same timing were recorded whatever the initial density) (Ellien et al. 2011). For both reasons, the exact quantity of prolarvae processed for this study was not taken into account.

Once they were collected, the clutches with their substrate were immediately transferred into two buckets filled with the water of the river. This transfer induced immediate hatching of the prolarvae.

The prolarvae were transported in the buckets to the laboratory within 3 h after their collect.

Once in the laboratory, two different protocols were followed, function of the period of the collect.

The prolarvae sampled in April 2010 were randomly distributed between two 45 l tanks, filled with freshwater (tap water) that was first aerated during 48 h before receiving the prolarvae. Bubbling in the tank oxygenates the water and also allows a decrease of the chlorides that are found in tap water in greater concentration than in the river freshwater. The prolarvae were maintained in these tanks, at an average density of 500 ind·l^{-1}, during 48 h before starting the experiment. The freshwater of the two tanks was entirely renewed in 24 h, with tap water previously oxygenated during 24 h. The room was not air-conditioned, so that the water temperature depended on the air temperature and was on average 26.9 °C. The tanks were located under electric light with a programmed 12 h day/night cycle.

To observe the influence of the sea water on the transformation of the prolarvae into marine larvae, the experiment consisted in transferring these prolarvae after 48 h into tanks filled with sea water. The same kind of tanks was used (i.e. 45 l tanks). They were filled with offshore drilling water circulating in open circuit, also passing previously through two degassing columns. Aeration took place directly in the tanks. This experiment was undertaken in duplicates, so two tanks of sea water were installed. A control consisting in two tanks (i.e. for the duplicate) filled with tap water previously aerated during 24 h was also installed, to observe the evolution of the prolarvae in strict freshwater. The prolarvae were transferred into the experimental and control tanks simultaneously, that is to say 48 h after their collect. In each tank, the prolarvae density at the beginning of the experiment was 100 ind·l^{-1}. Larvae were not fed during the experiment. During the experiment, the temperatures were on average 26.9 °C for the freshwater and 27.1 °C for the sea water. All tanks were located under the same programmed 12-h day/night cycle. Each day, the tanks were cleaned by siphoning off the dead individuals and waste material.

Ten prolarvae or larvae were randomly sampled every 1 h 30 on a period of 10 h per day and observed under a binocular lens set with a camera (Olympus C-5050; optical zoom x3; Olympus Ltd, Paris). The observation (including the photos) of each group of individuals lasted 10 min at the very most. The individuals were observed alive and not anaesthetised.

The prolarvae sampled between April and May 2012 and between April and June 2013 were randomly distributed between two 200-ml beakers (i.e. for the duplicates) filled with sea water, and two 200 ml control beakers (i.e. for the duplicates) filled with freshwater, once in the laboratory, immediately after the egg collect. The prolarvae were maintained in the different beakers at a density of 500 ind·l^{-1}.

For these experiments, the sea water was artificially synthesised by diluting the appropriate quantity of special Instant Ocean salt with freshwater (tap water), thus reproducing the ionic composition of sea water. This salty water was made at the average concentration of the Indian Ocean (i.e. 36.5). The obtained salinity was verified with a refractometer (Reichert, W2789H, Buffalo, NY, USA). The freshwater used for the control came from a river and was pipe-distributed (used only for field irrigation). It contains fewer chlorides than tap water. The freshwater used for both control (river freshwater) and fabrication of sea water (tap water) was previously aerated during 24 h. During the whole time of the experiment, the water was continuously aerated by bubbling in the beakers.

The laboratory was air-conditioned, so that the water temperature was maintained at 25.0 °C.

The four beakers were placed under electric light with a programmed 15-h/9-h day/night cycle.

Larvae were not fed during the experiment.

Fifteen prolarvae or larvae were observed twice a day, with an optical microscope (Olympus CX41, optical zoom ×40) set with a camera (Olympus E5).

For all experiments, prolarvae and larvae that have been sampled in the tanks or in the beakers for observation were considered as dead, so that they were not put back in their tank or beaker after observation.

Results

Larval development in freshwater

In freshwater, the morphological characteristics of the prolarvae show little development, whatever the time spent in this environment, within the 96 h of their observed maximal survival time, leading to the conclusion that the individuals remain at the prolarval stage as long as they do not reach the sea. Similar results were observed by Ellien et al. (2011), on the same species.

The prolarval stage is identified as L1 stage.

At hatching (stage L1a), prolarvae are characterised by the presence of the yolk sac, which is at its maximum size (around 0.3 mm). Pigmentation consists in four chromatophores located at the mouth, on the posterior part of the yolk sac, above the digestive tract and between the anus and the caudal fin. Eyes are located on lateral sides of the head, and they are not pigmented. There size reaches 0.1 mm on the longer length. Mouth and anus are closed. Except for the caudal fin, none of the fins are formed. The finfold presents a width of 0.04 mm. The size [i.e. total length (TL)] of the

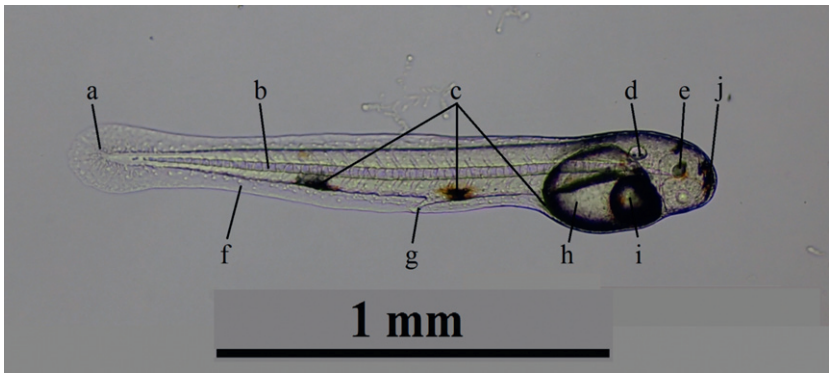

Fig. 2. Sicyopterus lagocephalus prolarva at hatching in freshwater. a: tip of notochord, b: notochord, c: chromatophores, d: otolith, e: eye, f: finfold, g: location of the anus, h: yolk sac, i: oil globule, j: location of the mouth.

individuals is always under 2 mm: their size is on average 1.7 mm (TL) (Fig. 2).

An ontogenetic development, slightly modifying the prolarval morphology, is observed. The yolk sac is partially resorbed (i.e. its size decreases to around 0.26 mm) after 28 h in freshwater at 25.0 °C and after 24 h at 26.9 °C, the oil globule becoming proportionally prominent (stage L1b), although the yolk sac is never entirely absorbed even when the prolarvae die in freshwater.

The pigmentation tends to spread horizontally along the digestive tract 24 h after hatching (stage L1b), to reach its maximum extension, although remaining in the shape of starry spots, after 50 h in freshwater at 25.0 °C, and after 48 h at 26.9 °C (stage L1c). The prolarvae remain translucent.

The lenses appear after 24 h at 26.9 °C (28 h at 25.0 °C) (stage L1b), and the eye pigmentation increases progressively. They reach their most intense colouration after 48 h in freshwater at 26.9 °C (50 h at 25.0 °C), without being entirely pigmented (L1c).

Whatever the time spent in freshwater (both at 25.0 and 26.9 °C), mouth and anus remain close, leading to the conclusion that the prolarvae do not feed.

Fins do not appear, whatever the time spent in freshwater and whatever its temperature.

No significant growth of the prolarvae is observed, so that they keep the same length than at hatching, namely nearly 2 mm (TL) (Fig. 3, Table 1).

In freshwater, prolarvae show a gregarious behaviour as soon as they hatch, gathering in columns of high densities in the central part of their container (i.e. buckets, tanks or beakers). Within these columns, they alternatively swim towards the surface and passively sink downwards, in a 'yo–yo' movement. This behaviour is observed for both temperatures.

Larval development in sea water

The transfer of prolarvae in tanks or beakers filled with sea water at a salinity of 36.5 induces major morphological modifications, characterising the marine larval stage. This marine larval stage is identified as L2 stage.

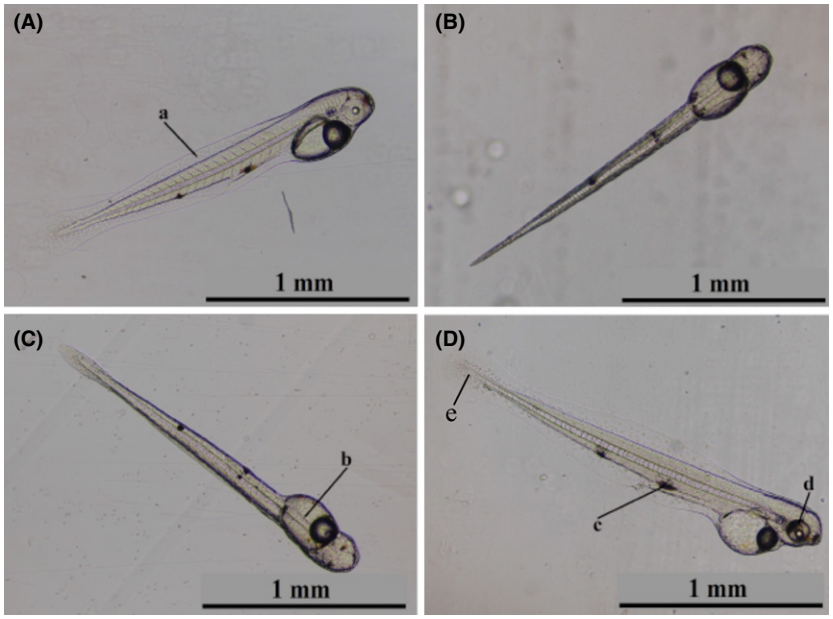

Fig. 3. Ontogenetic development of *Sicyopterus lagocephalus* prolarvae in freshwater at 25.0 °C. (A) 4 h after hatching, (B) 22 h after hatching, (C) 28 h after hatching, (D) 50 h after hatching. (a) finfold, (b) yolk sac, (c) chromatophores, (d) eye, (e) tip of notochord.

Table 1. Description of the prolarval characteristics and definition of the freshwater prolarval stages observed for *Sicyopterus lagocephalus* just after hatching and as long as they remain in freshwater.

	Freshwater at 25 °C	Freshwater at 26.9 °C	Stage
Appearance of the posterior tip of mandible, below the eye	18 h	15 h	L1b
Yolk sac partially resorbed	28 h	24 h	L1b
Pigmentation begins to extent	≈24 h	24 h	L1b
Pigmentation maximum extension	50 h	48 h	L1c
Appearance of the lenses	28 h	24 h	L1b
Eyes maximum pigmentation	50 h	48 h	L1c
Maximum size	≈2 mm	≈2 mm	L1b-c
All prolarvae died	Not recorded	96 h	

The first character undergoing a noticeable modification is the finfold that widens (i.e. its size increases until around 0.1 mm) as soon as the prolarvae are transferred in sea water at 27.1 °C. This modification becomes obvious after 4 h at 25.0 °C.

The yolk sac is rapidly absorbed until it consists in the oil globule only, after 22 h in sea water at 25.0 °C and 20 h in sea water at 27.1 °C.

Pigmentation spreads until it forms an almost continuous line above the digestive tract, after 50 h in sea water at 25.0 °C and after 48 h at 27.1 °C. Pigmentation also progressively extends on the cephalic part, as soon as they are transferred in sea water, to be fully pigmented after 50 h at 25.0 °C and after 48 h at 27.1 °C.

Eyes progressively migrate in anterolateral position of the head, 26 h after transfer of the prolarvae in sea water at 25.0 °C (after 24 h at 27.1 °C). They are entirely pigmented and functional (i.e. they are mobile, with prominent lenses) after 50 h at 25.0 °C and after around 40 h at 27.1 °C.

The mouth migrates in subinferior position of the head. Mouth and anus are open after 50 h in sea water at 25.0 °C, and after 48 h at 27.1 °C. The opening of the mouth goes with a morphological modification of the head, which appears more shaped because of the loss of the membrane covering the mouth. In parallel to the opening of the mouth and anus, the digestive tract thickens and peristaltic movements propagate from its anterior to its posterior part, showing that the digestive system is functional.

Pectoral fin buds appear after 6 h in sea water at 27.1 °C, and a bit more at 25.0 °C, to be entirely developed and functional after 48 h at 27.1 °C. However, this development is slower at 25.0 °C, and after 50 h, the pectoral fins are barely functional.

After 50 h at 25.0 °C (respectively 48 h at 27.1 °C), no larval growth is recorded: the larvae remain at a length of nearly 2 mm (TL) (Fig. 4, Table 2).

In sea water, larvae remain in a vertical position, their head orientated downwards, and keep on migrating up and down alternatively. However, they also progressively spend more time horizontally (i.e. up to 50% of their time), after their mouth opens, showing sudden accelerations in their swimming behaviour.

The prolarval and larval morphological characteristics are identical whether the observations were made in 2010, 2012 or 2013, the only difference being the duration of the transformation that varies according to the water temperature that was higher in 2010 than in 2012 and 2013.

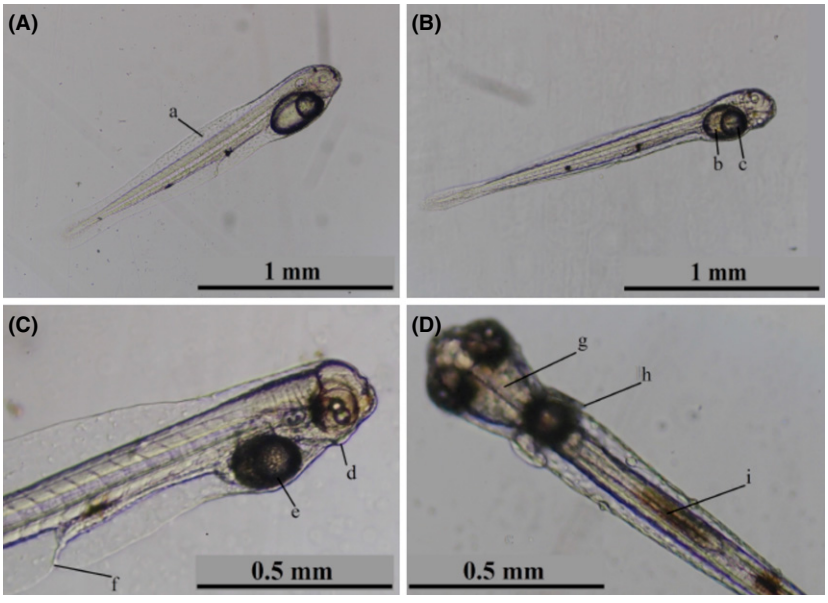

Fig. 4. Morphological development of *Sicyopterus lagocephalus* larvae in sea water at 25.0 °C. (A) 4 h after transfer in sea water, (B) 22 h after transfer, (C) 28 h after transfer, (D) 50 h after transfer. (a) finfold, (b) reduced yolk sac, (c) oil globule, (d) jaw, (e) yolk sac entirely absorbed, with oil globule prominent, (f) opened anus, (g) shaped head that becomes pigmented, (h) pectoral fin buds, (i) chromatophores.

Table 2. Description of the larval characteristics and definition of the marine larval stages observed for *Sicyopterus lagocephalus* after their transfer is sea water, at two distinct temperatures (25 and 27.1 °C).

	Sea water at 25 °C	Sea water at 27.1 °C	Stage
Finfold enlargement	4 h	Immediate	L2a
Yolk sac fully resorbed	22 h	20 h	L2a
Migration of the eyes in anterolateral position of the head	26 h	24 h	L2a
Pigmentation extended in a continuous line	50 h	48 h	L2b
Head pigmented	50 h	48 h	L2b
Eyes pigmented and functional	50 h	40 h	L2b
Mouth and anus open	50 h	48 h	L2b
Morphological modification of the head that appears more shaped	50 h	48 h	L2b
Digestive tract functional	50 h	48 h	L2b
Appearance of pectoral fins buds	7–8 h	6 h	L2a
Pectoral fins fully functional	+50 h	48 h	L2b
All larvae died	Not recorded	120 h	

Discussion

A need for a new description of *S. lagocephalus* prolarval and larval stages and substages

In Reunion Island, *S. lagocephalus* freshwater stages have been studied for two decades, for the juvenile and adult stages, but the description of the freshwater prolarval stages and of the first marine larval stages is recent (see Valade et al. 2009), although of major importance. However, our own observations of these developmental stages, undertaken repeatedly between 2010 and 2013, revealed some crucial discrepancies with this published description (Table 3), highlighting that a rigorous and accurate description of the morphological modifications, characterising the transformation of the freshwater prolarvae into marine larvae, is required, to understand the physiological changes.

Concerning the early development in freshwater, Valade et al. (2009) did not see any apparent modification of the prolarval morphology, defining this entire freshwater stage as the prolarval stage, identified as L1a. However, our observations do not lead to the same description. Indeed, the L1a stage described by Valade et al. (2009) corresponds to the prolarval morphology at hatching only, with the difference that Valade et al. (2009) did not see any trace of eye, while eyes are well visible in our observations, even if they are translucent (Fig. 2, Table 3). Yamasaki & Tachihara (2006) observed the same morphological modifications pattern on *Stiphodon percnopterygionus* (Gobiidae: Sicydiinae) larvae reared during 1 day in freshwater before their transfer in sea water. These authors observed the presence of lens on newly hatched larvae even if the eyes were not pigmented and also the presence of melanophores on different parts of the larval body. During this first day in freshwater, slight modifications occur, concerning mostly the size of the yolk sac that decreases with time (Yamasaki & Tachihara 2006). The authors transferred the larvae in salty water (i.e. salinity of 25) at the end of the first day after hatching, so that further observations of the larvae in freshwater did not occur. Iida et al. (2013) described *S. japonicus* larvae at hatching and noted the presence of unpigmented although visible eyes, a developed yolk sac and unopened mouth. Bell (1994) observed larval morphology and development of *Sicydium punctatum* (Gobiidae: Sicydiinae) and described larvae at hatching or recently hatched, at development stages from no eye structure visible to eye with lens and some retinal pigments. However, larvae with no visible eye structure being rare, the author concluded that this larval stage hatches prematurely or under unusual condition. Then, our observations on *S. lagocephalus* prolarvae are in good agreement with these previous descriptions, about the presence of the eyes at hatching, but also on the criterion of pigmentation increasing with time. Bell (1994) also showed that after 96 h in freshwater, larval yolk reserves still remained, but decreased in size (from 280 μm in diameter at hatching to 140 μm after 96 h), and jaw structures remained incompletely developed. Our observations on *S. lagocephalus* lead to the same conclusion, namely the occurrence of ontogenetic development occurring in freshwater that slightly modifies prolarval morphology as early as 18 h after hatching in freshwater at 25.0 °C (and 15 h at 26.9 °C) (Table 1). These morphological modifications, allowing describing the substages L1b and c, occur in strict freshwater (Fig. 3, Table 3). As Bell (1994) with *Sicydium punctatum* larvae, *S. lagocephalus* prolarvae survived up to 96 h in freshwater in our experiments.

Valade et al. (2009) observed prolarvae in freshwater tanks during a maximum of 72 h and saw no morphological modification of the prolarvae during this phase.

It is only after the transfer of prolarvae in sea water that Valade et al. (2009) observed the same modifications that we observed in freshwater (Table 3). In particular, according to Valade et al. (2009), the yolk sac begins to decrease and the lenses appear, in sea water, 28 h after hatching for a water temperature of 25.0 °C. The posterior tip of the mandible is detectable below the eyes 18 h after hatching. All these modifications are described in Valade et al. (2009) after prolarvae spent between 0 (i.e. larvae just arriving at sea) and 40 h in sea water.

Table 3. Summary of the prolarval and larval morphological descriptions found in Valade et al. (2009) vs. the new description proposed in this study. The reference stages are indicated beside the corresponding morphological description. The cells in grey indicate the observations made after the transfer of the prolarvae in sea water.

Previous description (Valade et al. 2009)	Stage	New description based on this study	Stage
No trace of eye	L1a	Yolk sac at its maximum size	L1a
No sign of jaw		Pigmentation reduced to four chromatophores	
Translucent		Eyes translucent on lateral sides of the head	
Yolk sac at its maximum size		Mouth and anus closed	
Early eyes (no lens or lens unpigmented) on lateral sides of the head	L1b	No sign of jaw	
Posterior tip of the mandible detectable as a prominence below eye		No fin (except caudal fin)	
Beginning of yolk sac absorption			
Lens present and pigment beginning to appear on retina	L1c	Yolk sac partially resorbed	L1b
Beginning of the migration of the eyes in anterolateral position of the head		Appearance of the lenses	
Mouth formed but closed		Appearance of the posterior tip of the mandible detectable as a prominence below eye	
Appearance of pectoral fins		Pigmentation at its maximum extension	L1c
Multiplication of chromatophores on the body		Eyes maximum pigmentation	
Lens pigmented	L1d	Finfold enlargement	L2a
Eyes in anterolateral position of the head		Yolk sac fully resorbed	
Mouth open		Appearance of pectoral fins buds	
Pectoral fins in place		Migration of the eyes in anterolateral position of the head	
Yolk sac same size as the eyes		Pigmentation extended in a continuous line	L2b
Appearance of chromatophores in cephalic area		Head pigmented	
Lens pigmented	L2	Eyes (and lenses) pigmented and functional	
Mouth well formed open in terminal position and operating		Mouth and anus open and functional	
Ability to intake and digest exogenous food		Digestive tract functional	
No more yolk sac		Modification of the head that appears more shaped	
Many chromatophores in the cephalic area and along the body		Pectoral fins functional	
All internal organs in place			

Following our observations, the transfer of prolarvae in sea water induces important morphological modifications (Table 2), justifying the definition of the L2 stage that corresponds to the larval marine stage. Within this stage, we define two substages: L2a stage that corresponds to the organisation of the morphological structures allowing the organisms to adapt to their new marine environment (e.g. appearance of the pectoral fins buds, migration of the eyes in anterolateral position of the head, finfold enlargement that constitutes the beginning of the fins formation) and L2b stage during which these morphological structures become functional (e.g. eyes functional, mouth and anus open and pectoral fins functional. . .). The L2b stage is set up two folds later than the L2a stage (Table 2). The difference between the L1 and L2 stages is not clearly detailed in Valade et al. (2009), so that redundancies occur, for example between the stages L1d and L2 that are both characterised by lens pigmented, mouth open in terminal position and chromatophores in the cephalic area.

The sequence in the morphological modifications characterising the passage from freshwater prolarvae to marine larvae, as described in the present study, reveals to be quite similar in several other studies on sicydiine fish. Indeed, Iida et al. (2010) observed on *S. japonicus* larvae transferred in salty water, eye pigmentation, mouth opening and yolk absorption, whatever the salinity of the salty water, whereas these modifications did not occur in freshwater. Similarly, Yamasaki & Tachihara (2006) described the acquisition of the marine characters on *Stiphodon percnopterygionus* larvae after their transfer in salty water at a salinity of 25, namely appearance of pectoral fins, modification of the body and cephalic pigmentation, opening of the mouth and anus and resorption of the yolk sac. Again Yamasaki & Tachihara (2007) highlighted the same pattern in the morphological modifications undergone by *Awaous melanocephalus* (Teleostei: Gobiidae) larvae once in salty water (i.e. 50% sea water and 50% freshwater).

In Reunion Island, according to the river length (i.e. 36 km maximum) and the river flow intensity that reaches an average speed of 5 $km \cdot h^{-1}$ (Mérigoux et al. 2009; Teichert et al. 2014), prolarvae are transported downstream to the sea in <24 h (i.e. in 7 h on average), whatever the clutch location in the rivers. In this study, we observed that prolarvae were able to survive up to 96 h in freshwater, confirming a previous study leading to the same conclusion (Ellien et al. 2011). Thus, theoretically *S. lagocephalus* prolarvae reach the sea before undergoing most of the ontogenetic modifications that occur in freshwater.

However, it is important to know that these modifications are possible, associated to a survival of 96 h in freshwater, in case a delay in the prolarval downstream transport occurs (e.g. exsiccation periods downstream of the rivers, dam construction slowing down the river flow intensity).

Furthermore, even if *S. lagocephalus* prolarvae show little ontogenetic development in freshwater, they need to reach the sea as fast as possible to pursue their development. In particular, eyes are not functional in freshwater, even though the pigmentation increases in 2 days after hatching. This absence of functional eyes and their poor ontogenetic development makes the prolarvae easy preys, as they seem unable to avoid their predator. Then, *S. lagocephalus* prolarvae need to reach the sea in a narrow temporal scale of 4 days, at a water temperature ranging from 20 to 26.9 °C, otherwise they die, probably of physiological causes (i.e. osmoregulation). Their yolk sac being still present, even though partially resorbed, starvation does not seem to be a mortality factor.

Influence of water temperature on prolarval and larval development

Temperature is known to influence larval development of many teleosts on which it has been studied (see in particular for sicydiine fish: Iida et al. 2010; Valade et al. 2009). The warmer the temperature, the faster the development. Our present study confirms this observation, as we have shown that at a higher temperature, the morphological modifications of *S. lagocephalus* prolarvae and larvae take place faster than at a cooler temperature, in freshwater (25.0 °C vs. 26.9 °C) as well as in sea water (25.0 °C vs. 27.1 °C). However, whatever the temperature, the development of *S. lagocephalus* prolarvae in freshwater and their transformation into marine larvae follow the same pattern and the same chronology, with a delay of few hours in the timing of appearance of the characteristic structures, when the temperature is cooler of ±2 °C. Similarly Borges et al. (2003) have shown that embryonic development of the Gobiidae *Gobius paganellus* lasted 9–10 days at a temperature of 18.5–20 °C and 10–11 days at a temperature of 15–16.5 °C, yet conserving the same sequence of development between the temperature ranges. Iida et al. (2010) observed also the same influence of temperature on larval development for the Sicydiinae *S. japonicus*, with higher temperatures allowing faster development. For *S. lagocephalus*, Valade et al. (2009) highlighted that sea water temperature has an influence on larval development which is faster at 26 °C than at 22 °C, even if the sequence of embryonic development remains similar between temperatures. All these studies converge towards the same observations concerning the influence of temperature on diadromous fish larvae development.

Indeed, temperature is known to act on metabolism, by accelerating it when temperature is higher. The results of our study are in good agreement with this theory. In Reunion Island, the water temperature in the Langevin River, where the clutches were collected, ranges between 20 and 23 °C during the main reproduction period (i.e. January to June), thus lower than the temperatures tested during our experiments. Then, we can assume that prolarval development is slower in these natural conditions than what we observed experimentally. However, for freshwater temperature between 20 and 23 °C, Valade et al. (2009) observed, for *S. lagocephalus* prolarvae, a maximum survival duration between 3 and 4 days, that is to say not different from what we observed for prolarvae in freshwater at 26.9 °C (96 h). Then, if prolarval development is slowed down in colder temperature, their survival does not seem significantly improved.

Conclusion

This study proposes a rigorous and accurate description of the morphological modifications, characterising the transformation of the prolarvae into larvae during their passage from freshwater to sea water, according to different temperatures in freshwater as well as in sea water. The main observations are in good agreement with previous studies on amphidromous gobies in other regions (e.g. Yamasaki & Tachihara 2006; Iida et al. 2010) concerning the prolarvae developmental stage at hatching as well as the early developmental sequence in freshwater. In all cases, Sicydiinae species are characterised by poorly developed freshwater prolarval stages. Even if *S. lagocephalus* prolarvae show a poor but visible ontogenetic development in freshwater, they need to reach the sea in a narrow temporal scale of 4 days, at a water temperature ranging from 20 to 26.9 °C, to pursue their development, otherwise they die probably of physiological causes (i.e. osmoregulation). In this context, any obstacle in the river/sea corridor (e.g. exsiccation periods, construction of dams…) delaying their arrival at sea is lethal for the prolarvae and prejudicial for the wealth of the stock, already damaged by overfishing on recruiting postlarvae.

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