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Ontogeny of the digestive and feeding systems in the anemonefish *Amphiprion melanopus*

Bridget S. Green & Mark I. McCormick
Department of Marine Biology, James Cook University, Townsville, Queensland, 4812 Australia
(e-mail: bridget.green@jcu.edu.au)

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Synopsis

Ontogenetic growth and development in the anemonefish *Amphiprion melanopus* (Pomacentridae) is very rapid when compared to other tropical and temperate fish species. *A. melanopus* hatched with a highly differentiated digestive tract and the ability to capture and ingest prey items. Their alimentary tract changes rapidly throughout the larval period. Concurrent with yolksac absorption at three days after hatching was the development of the stomach followed by calcification of the jaw structures. This period of acute structural change may be a critical period in their development. Metamorphosis coincided with settlement at 8 days after hatching and was marked by calcification of fins and acquirement of adult coloration. The rapid development found in this species may be a specialisation to enhance the return of larvae to a patchy and highly specific settlement habitat.

Introduction

For many fish there are 'critical periods' in development whereby they must reach certain functional thresholds in order to procure and digest food (Hjort 1914, Houde 1974, Blaxter 1988, Thorisson 1994). The onset of exogenous feeding represents a period of high mortality for fish larvae, particularly those in tropical and sub-tropical waters, where the transitional period between yolk absorption and first feeding is short (Houde 1974, Bagarinao 1986, Houde & Zastrow 1993). Fish larvae must be competent at procuring and assimilating food before the yolksac and oil globule are depleted, or risk starvation (Avila & Juario 1987, Fukuhara 1988). A second 'critical period', at the end of the larva period during the transition to a benthic juvenile form, has been proposed for demersal fish species (Blaxter 1988). For many species a complex metamorphosis occurs at this time involving rapid changes in morphology (e.g. Markle et al. 1992), sensory systems (e.g. McCormick 1993, Shand 1994, Job & Bellwood 1996), locomotory ability (e.g. Fukuhara 1985), respiration (e.g. Hunt von Herbing et al. 1996a) and digestive and hormonal physiology (Walford & Lam 1993). This is also a time when a fish must exploit new food sources and avoid new predators. The rate of development is likely to determine the survival of the fish as it undergoes these physical and ecological changes. However, currently very little information is available on the changes that occur in the digestive mechanisms and feeding structures of tropical reef fishes during ontogeny and the settlement transition.

Studies on the ontogeny of fish larvae have often focused on only one area of development, either osteological (e.g. Potthoff et al. 1987, Potthoff & Tellock 1993) or changes in the digestive system (e.g. Kurokawa et al. 1995). However an integrated consideration of different structures during ontogeny is important to understand how structural changes may influence functional capability as the fish changes environments. The intestinal tract must grow and differentiate with changes in the feeding ecology associated with first feeding and metamorphosis. The skeletal system of fish must grow and ossify to provide leverage for feeding and swimming as the

fish becomes active in prey capture and predator avoidance.

The present study documents the changes in internal and external morphology that occur during the larva period and metamorphosis in the tropical reef fish *Amphiprion melanopus* (Pomacentridae). In particular it focuses on the changes associated with the head and alimentary tract, including a histological examination of changes in cell types and gut differentiation and an examination of the morphology and calcification of bone structures, including the feeding structures, vertebral column and the fins.

Material and methods

Rearing

Amphiprion melanopus were reared in an indoor laboratory at the James Cook University aquarium facility. Adult *A. melanopus* were provided with a terracotta pot as artificial substratum on which to lay their eggs. Eggs were laid in clutches of 500–1000 and took approximately 8–10 days to develop. After dusk, a single clutch of full-sibling embryos was hatched into a 140 litre glass tank where temperature was maintained at $28^{\circ}C \pm 1^{\circ}C$. Air passing through an airstone maintained water circulation. The tank was coated in an external black gel-coating to minimise light entering the sides of the tank and reduce disturbances from the laboratory. A culture of *Nannochloropsis* sp. algae was added to the tank each morning at a density such that the airstone on the bottom of the tank was not visible (emulating a secchi disc). The algae were required to diffuse light, increase contrast for feeding and act as food for the zooplanktonic prey items (Naas et al. 1992) and reduce reflections off the aquarium that cause 'head-banging' behaviour (fish larvae will swim into the sides of the tank, banging their heads until they die). Fluorescent lights were used to simulate a 14 h summer daylight cycle. The hatching tank was maintained as a semi-closed system that was flushed nightly when the lights were off. Salinity was maintained between 33 and 34.7‰.

On the first morning after hatching *A. melanopus* larvae were fed rotifers (*Brachionus* sp.) at a density of ~ 5 ml^{-1}, boosted with yeast (Daintith 1993) and *Nannochloropsis* sp. algae. On the third day after hatching *Artemia* nauplii were added, and from day 4 after hatching onwards fish were fed only on *Artemia* at a density of 1–2 ml^{-1}.

Sampling

Ten larvae were sampled every day immediately after feeding, until day 9 after hatching. Sampled fish were placed in vials of seawater and put on ice until dead. Five fish from each day's sample were fixed in 10% salt-water formalin (Borax-buffered) for clearing-and-staining, and five fish were fixed in Marine Bouin's for histology. All specimens were transferred to 70% alcohol after two to three hours in fixative to avoid the loss of bone mineral in specimens prepared for clearing and staining (Taylor & Van Dyke 1985). Standard length was measured on all fish prior to histological processing using a stereo-dissecting microscope equipped with an ocular micrometer and a stage graticule calibrated to the nearest 0.12 mm. Standard length was adjusted for shrinkage using regression equations (Green & McCormick 1999). Whole dissections were performed on at least one fish per day to confirm the stages of organ development.

Osteological examination

Fish for osteological examination were cleared and double-stained following the methods of Dingerkus & Uhler (1977) with a modification on the enzyme used in the clearing process (Gosztonyi 1984). Trypsin was replaced with a cheaper and more readily available alternative 'Preen' brand enzyme-based laundry presoak[1]. Alcian blue is specific for mucopolysaccharides, and therefore stains cartilage dark blue, while the counterstain, alizarin red S, stains bone and calcified structures red. Specimens were examined microscopically and using photomicrographs to identify the appearance of the stains identifying cartilage and bone mineral. Blue structures were considered to be cartilage while any appearance of pink or red was taken as an indication of bone mineral and therefore calcification.

Histological examination of the gut

Fish for histological examination were processed using standard histological techniques (Windsor 1994). Specimens were mounted in paraffin wax blocks and serially sectioned in the sagittal plane at 5 μm,

[1] N.B. This has since been repeated and 'Preen' is no longer suitable as a replacement. The authors assume that the chemical composition has been altered.

mounted on microslides and stained with Mayer's alum haematoxylin and Young's eosin–erythrosin, and dried in an oven at 40°C for at least one week. Sections were then examined under a high power microscope at 400× magnification and all cells and organs were examined. Particular attention was paid to the epithelium cells lining the alimentary tract as this is where gross cellular differentiation occurs (e.g. Govoni 1980).

Results

Osteological development

1 day after hatching (1 dah) (mean standard length (SL) – 4.17 mm). All fundamental skeletal elements were present, albeit in a rudimentary form, flexion was complete although skeletal structures showed no signs of calcification. Pectorals fins were present, small fin rays had formed, however no spines were present. Epurals and procurrent caudal rays were present (Figure 1a). All skull structures are present, except no teeth were visible (Figure 2a). Tables 1 and 2 describe the extent of development.

2 dah (mean SL – 4.34 mm). Larvae still had no visible calcification. Pterygiophores for dorsal and anal fins were fully formed, although the pelvic and pectoral fins were still rudimentary. Two teeth were visible on the dentary.

3 dah (mean SL – 4.67 mm). The spinal cord was beginning to calcify at the anterior end, although all fins were still cartilaginous. The dorsal fin had 27 very short, undifferentiated rays, and the anal fin had 17 undifferentiated rays (Figure 1b).

4 dah (mean SL – 4.99 mm). Calcification extended mid-way down vertebral column, however vertebral ribs had no apparent calcification. Dentary, articular, branchiostegals, parietals, frontals and cleithrum were calcified, whilst the quadrate, pre-maxilla and maxilla were only partially calcified. There were a few teeth visible on the pre-maxilla and dentary. The hypurals, epurals, pectoral and pelvic fins still appeared cartilaginous.

5 dah (mean SL – 5.02 mm). The vertebral column was calcified its entire length, including spines and arches. Hypurals, caudal, pectoral and pelvic fins were only calcified at the proximal ends. The dorsal and anal spines still appeared cartilaginous. The dentary, articular, pre-maxilla and maxilla were calcified and many teeth were visible on the pre-maxilla and dentary

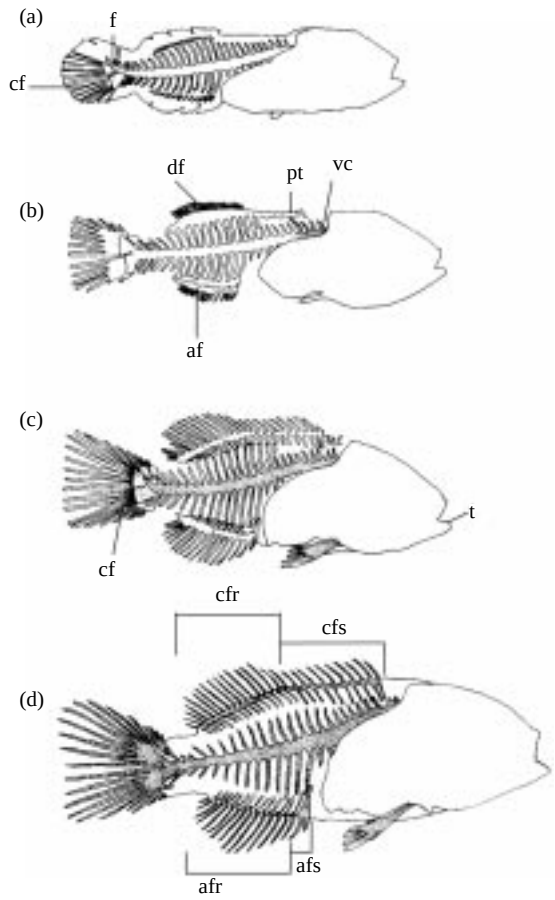

Figure 1. Representative developmental series of cleared and stained specimens of *Amphiprion melanopus* at 4 stages of the larva period. Specimens were stained with alizarin red and alcian blue. Stippling represents the presence of alizarin red: a – 1 dah, b – 3 dah, c – 6 dah, d – 8 dah (pf = pectoral fin, bs = branchiostegals, f = flexion, cf = caudal fin, df = dorsal fin, pt = pterygiophores, vc = vertebral column, af = anal fin, t = teeth, dfr = dorsal fin rays, dfs = dorsal fin spines, afr = anal fin rays, afs = anal fin spines). Scale bar – 1 cm.

(Figure 2b). The length of the snout had increased compared to 1 dah and the skull has enlarged dorsoventrally (Figure 2a cf. 2b).

6 dah (mean SL – 5.5 mm). Skeletal structures in the head were completely calcified. Dorsal and anal rays were calcified, with meristic counts of 9 and 2 respectively. Calcification in the caudal fin is extending distally, the vertebral ribs and spines were calcified entirely, and the 1st pterygiophore of the anal fin had calcified (Figure 1c).

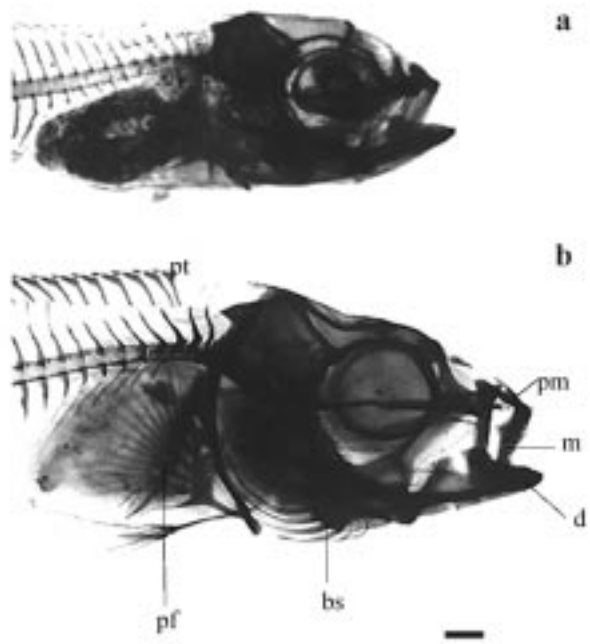

Figure 2. Cleared and stained specimens of *Amphiprion melanopus* showing development of the jaw structure at 2 stages in the larva period: a – 1 dah, b – 5 dah. Abbreviations as for Figure 1 (d = dentary, m = maxilla, pm = pre-maxilla). Scale bar – 0.2 mm.

7 dah (mean SL – 5.84 mm). Caudal calcification is increasing distally, the base of pelvics were calcified, but fin-rays still appear cartilaginous. Dorsal fin and anal fin spines also still appear to be cartilaginous. The opercular series had calcified.

8 dah (mean SL – 6.14 mm). Dorsal and anal spines and rays, and the caudal fin were calcified. Pterygiophores for rays were still cartilaginous, those for spines were distinctly calcified (Figure 1d).

9 dah (mean SL – 6.16 mm). The larvae had full adult meristic counts for dorsal and anal fins: D X, 18, A II, 15.

Histological development of the gut

1 dah (mean epithelium cell height 15.99 μm). Embryos were hatched with an eosinphilic yolksac and an oil globule. The gut was already differentiated into foregut, midgut and hindgut (as defined by the cell types lining these regions), – pseudostratified epithelium lined the midgut and hindgut, and the foregut was lined with cuboidal epithelium and contained mucous glands at the anterior end (Figure 3). There was also a muscular sphincter dividing the foregut from the midgut. The mucosal epithelium of the intestine was highly convoluted along its length. Vacuoles

Table 1. Summary of the development of calcification of feeding and locomotory structures of laboratory reared *Amphiprion melanopus* (*denotes presence, **denotes beginning of calcification, ***denotes completely calcified, dah – denotes days after hatching).

Structure	Timing								
	1 dah	2 dah	3 dah	4 dah	5 dah	6 dah	7 dah	8 dah	9 dah
Notochord flexion	*								
Cleithrum	*			***					
Quadrate	*		*	**	***				
Dentary	*			**	***				
Articular	*			**	***				
Pre-maxilla	*			**	***				
Maxilla	*			**	***				
Teeth		*		**					
Branchiostegals	*			***					
Opercular series	*						***		
Vertebral column	*		**	**	***				
Pectoral and pelvic fins	*				**	**		***	
Caudal fin	*				**			***	
Dorsal fin	*					**	**	***	
Anal fin	*					**	**	***	

Table 2. Summary of the major organ development of *Amphiprion melanopus* (*denotes presence, **denotes presence in more developed form, dah = days after hatching).

Feature	Timing			
	1 dah	2 dah	3 dah	4 dah
Yolksac	**	*		
Kidney	*			
Liver	*			
Pancreas	*			
Gut looped	*			
Stomach			*	**
Feeding	*			

(mucous-secreting goblet cells) were present in the hindgut (Figure 3c). The liver was present, however, it was very compact and granular and the hepatocytes lacked vacuoles (Figure 3b). Pancreatic tissue was present in areas surrounding the intestine, but was also very compact (Figure 3a). A kidney with tubules was present (Figure 3b). The gut was already looped in a single loop, there was a large intestinal lumen present and food was visible inside the lumen. Fish were observed feeding in aquaria prior to sampling.

2 dah (mean cell height 18.68 μm). The yolksac was still present. The only changes apparent in the gut were further differentiation of cell types in the anterior (foregut) section. Simple squamous epithelium lines the oesophagus until an aggregation of mucous secreting glands, then the epithelium is cuboidal and more rugose, which suggests that the cuboidal part of oesophagus will differentiate into the stomach.

3 dah (mean cell height 19.9 μm). The yolksac was fully absorbed. The hindgut was pseudostratified with diverticular forming in the mucosal layer. The stomach was visible as a slight enlargement of the oesophagus, with a sphincter at either end, and lined with cuboidal epithelium with many secretory cells. In a whole dissection this appears as a round, white organ, between the oesophagus and the intestine. The liver was compact and dense.

4–7 dah. The liver and pancreas had many large vacuoles where presumably lipids and glycogen were stored. The size of the vacuoles increased in size daily. The epithelium in the mid- and hind-gut was ciliated columnar, and contained many secretory/storage cells, while the cells lining the oesophagus varied along its length. The stomach was enlarging, and contained larger and more numerous secretory cells (Figure 4).

8 dah (mean cell height 26.5 μm). Numerous diverticular were present in the foregut, surrounded by mucous secreting cells. The oesophagus was lined with cuboidal epithelium and had a distinct glandular section anterior to the stomach. Settlement occurred at these stages, and probably metamorphosis, as defined by the development of full adult pigmentation.

Overall developmental summary

At hatching the fish appear to be without any skeletal calcification. Absorption of the yolksac, and the concurrent requirement to rely entirely on exogenous food sources coincided with calcification of the jaw structures and appearance of the stomach. This was quickly followed by calcification of the vertebral column and then the opercular series, which can be a secondary method of opening the jaw, and finally a rapid development of the fins prior to settlement.

Discussion

Gut development and its implications

As the digestive and feeding systems develop sufficiently for prey capture and digestion, the embryos must subsist on a maternally derived yolksac. The degree of cross-over between the two processes can determine a fish's survival. In *A. melanopus* the cellular differentiation between the fore-, mid- and hind-gut, the presence of a pancreas, epithelium lining and liver and the occurrence of food in the gut (day 1) suggests that the alimentary canal is equipped to assimilate food well before complete yolksac absorption (3 dah). The liver and pancreas expand rapidly and contain vacuoles within 4 days of hatching indicating they are already functional areas of lipid and glycogen storage. Further, supranuclear vacuoles within the mucosal epithelium of the fore- and hind-gut suggest that lipid assimilation is occurring (see Govoni et al. 1986).

Digestion in fishes generally occurs in two steps: (1) components of the diet, such as lipids, are hydrolysed into smaller molecules by enzymes secreted into the lumen of the gut by the pancreatic tissue. (2) Further hydrolysis of fragments of proteins, carbohydrates and lipids occurs on the mucosal surface of the intestine, and then within the mucosal cells or within the hepatocytes (Hofer 1991), lipids are resynthesised and deposited as lipid droplets within the mucosal epithelium cells (Govoni et al. 1986). The large intestinal lumen present in *Amphiprion melanopus* immediately after hatching suggests a large food storage

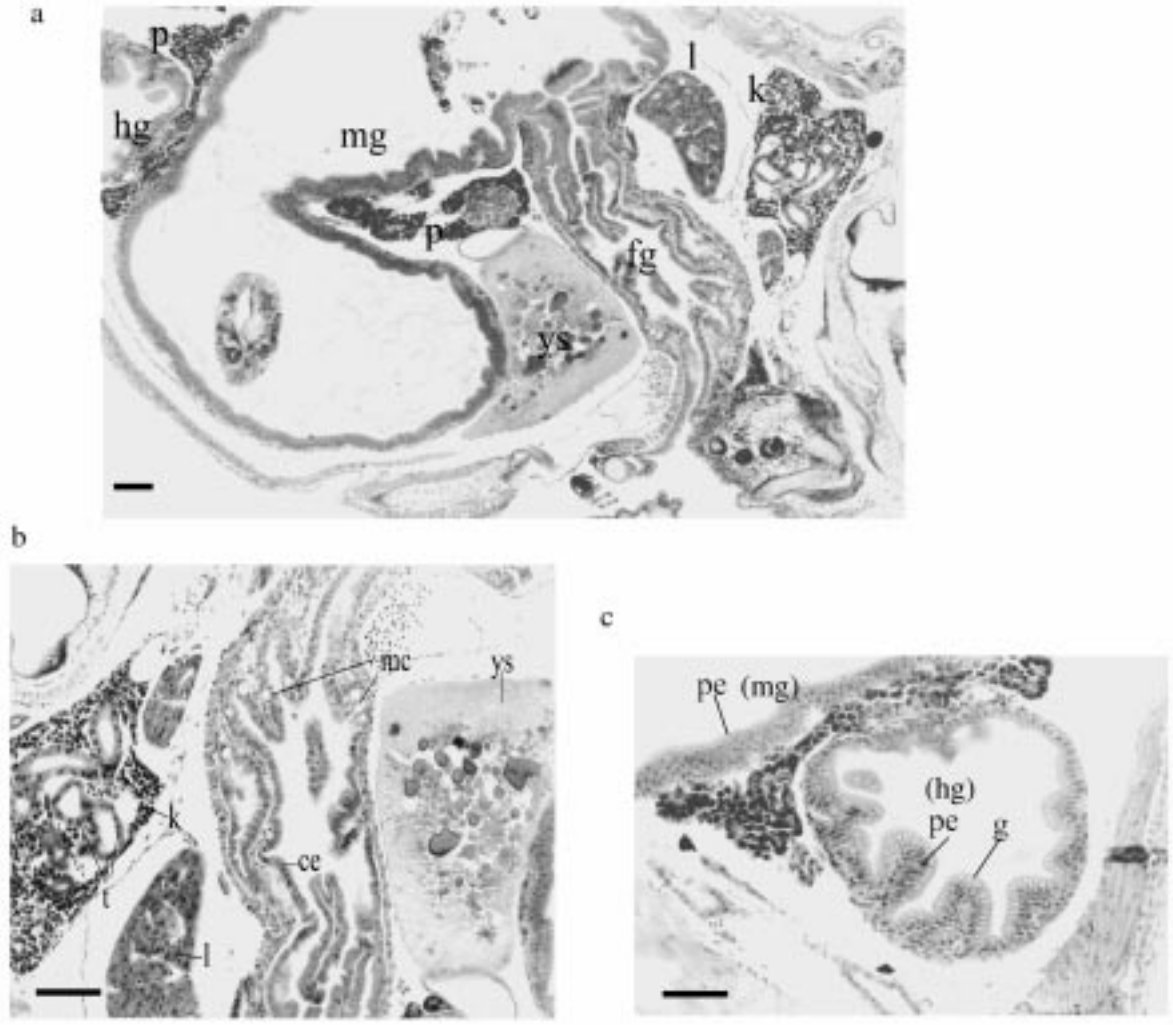

Figure 3. Histological section of the gut of *Amphiprion melanopus* on the first day after hatching. Sectioned at 5 μm, stained with Mayer's haemotoxylin and eosin: a – entire gut, b – foregut, liver and kidney, c – hindgut and pancreas (p = pancreas, hg = hindgut, mg = midgut, ys = yolksac, fg = foregut, l = liver, k = kidney, t = tubules, ce = cubiodal epithelium, mc = mucous cells, pe = pseudostratified epithelium, g = goblet cells). Scale bars – 50 μm.

capacity, which is advantageous in oceanic waters where encountering prey might be periodic (Govoni 1980, Mackenzie & Leggett 1991), and also provides a large area for digestive processes, such as lipid hydrolysis.

The stomach increased rapidly in size as the larvae grew, which in other species is concurrent with an increase in pepsin-like enzymes (e.g. *Lates calcarifer*, Walford & Lam 1993). In addition, mean gut epithelium cell height increased with age and size, increasing the surface area available for enzyme secretion and hydrolysis of nutrients throughout ontogenetic development.

Skeletal development and its implications

Jaw development was complete by day 4 after hatching in *Amphiprion melanopus*. All the primary bones were calcified and the articulations were present at these

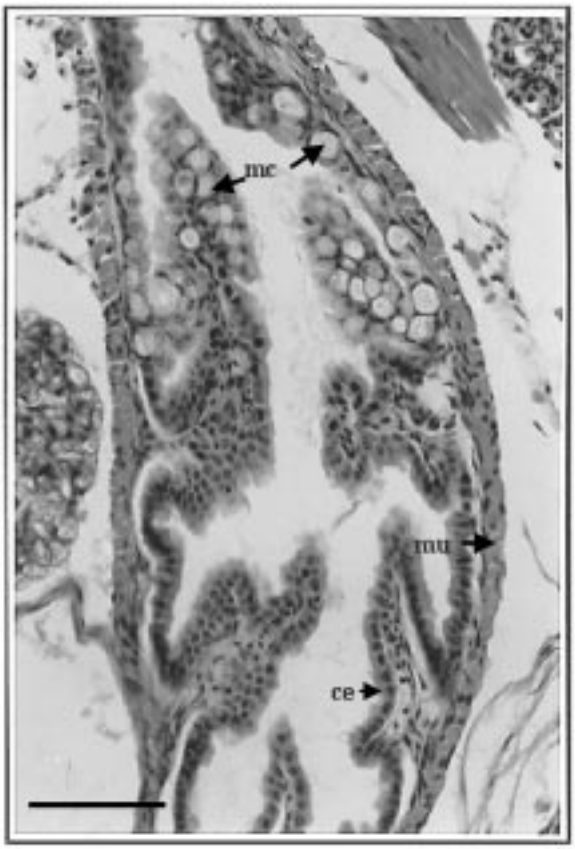

Figure 4. Histological section of the developing stomach of *Amphiprion melanopus* at 4 dah. Sectioned at 5 μm, sained with Mayer's haemotoxylin and eosin (mc = mucous cells, mu = muscularis, ce = cuboidal epithelium). Scale bar – 50 μm.

stages, although the opercular series was not fully calcified until day 7. The sequence of calcification and establishment of articulations that fish of this genus undergo suggests a functional relationship to their feeding ecology and food capturing processes (assuming that calcification increases ability). Prey capture success in the amprionine pomacentrids studied to date is high 3 days after hatching. For example, Coughlin (1994) found that *Amphiprion perideraion* had 100% strike success at day 3 and day 5 after hatching, while Job & Bellwood (1996) found that *Premnas biaculeatus* had 96% strike success at day 3 and 100% at day 10 after hatching. In comparison, 2 slower developing species with larvae of similar size had a much lower strike success. The common carp *Cyprinus carpio*, had 51% success at 2 dah and 88.5% success at 5 dah (Drost 1987) whilst anchovy larvae, *Engraulis mordax* had only 10% strike success at 3 days after hatching (Hunter 1972).

As head development occurs, the *Amphiprion* buccal cavity is transformed from a cylindrical to a truncated conical cavity (Liem 1991) which is thought to allow larvae to change to the more efficient suction feeding mode (Liem 1991) by rapidly expanding the cone-shaped buccopharyngeal cavity (Coughlin 1991). The *Amphiprion* pectoral fins, although still quite primitive at 5 days after hatching, are used in a sculling motion to move towards the prey (Coughlin 1994 and this study). It is the combination of this swimming motion and the well-developed orobranchial chamber that is thought to be responsible for their high capture success (Coughlin 1994).

Development relative to other species

The sequence of developmental events for *Amphiprion melanopus* is similar to the sequence of development described for many other fish species (e.g. *Microspathodon chrysurus* Pomacentridae, Potthoff et al. 1987; *Thunnus thynnus* Scombridae, Kaji et al. 1996; *Gadus morhua* Gadiformes, Hunt von Herbing et al. 1996a), however, occurs more rapidly (Table 3). Temperature is often a key factor in developmental differences (Hunt von Herbing et al. 1996b), however 6 species in Table 3 had similar temperature regimes to *A. melanopus* but their development was slower. For example, the milkfish, *Chanos chanos*, does not feed until 5 days after hatching (Bagarinao 1986), and *Lates calcarifer* does not open its mouth until the 2 days after hatching (Walford & Lam 1993).

A. melanopus hatch with the ability to feed, swim and catch prey. In contrast, many temperate species hatch without a through gut, and no mouth or anus (Table 3) (e.g. *Hippoglossus hippoglossus*, Blaxter et al. 1983). Based on the slower development of temperate fish, it has long been held that most marine fish cannot feed immediately upon hatching (Houde 1974) and have a straight incipient gut until settlement (Govoni et al. 1986). However *A. melanopus*, which have a highly differentiated intestinal tract, were seen to feed and had food present in their stomach on the first day after hatching. Unlike many temperate fish (e.g. anchovies, sea bream and lined sole, Houde 1974), *A. melanopus* initiate feeding days prior to the completion of yolksac absorption. In most of the temperate species studied

Table 3. Timing of development in some marine fish larvae from different orders. All timing in days after hatching unless otherwise noted (b = benthic egg, p = pelagic egg, h = hours, d = days).

Species	Mouth open	First feeding	Yolk absorption	Gut differentiation	Egg duration	Flexion	Larva duration	Temp. °C	Reference
Perciformes									
Amphiprion melanopus	0	12 h	3	12 h	8 d (b)	0	8	28	This study
Siganus guttatus	24–36 h	2–3	3	1	20–26 h (b)		24	26–30	Bagarinao (1986), Avila & Juario (1987), Juario et al. (1985)
Lates calcarifer	1–2	2	2–5	1–8	14 h (p)		∼15	26–32	Bagarinao (1986), Avila & Juaria (1987), Walford & Lam (1993)
Thunnus thynnus	2	3	3	3	(p)	14	30	25	Kaji et al. (1996)
Sphyraena borealis	3	3	4		>20 h (p)	∼12	20–22	23.2–24.5	Houde (1972)
Gonorynchiformes									
Chanos chanos	54 hph	4	5		24 h (p)			26–30	Bagarinao (1986)
Pleuronectiformes									
Limanda yokohamae	1	2	6	∼60	8 d (b)	39	34–43	11.5	Fukuhara (1988)
Hippoglossus hippoglossus	21	30	50	40					Blaxter et al. (1983)
Paralichthys olivaceus	3	4	4		4 d (p)	35	80–90	15	Fukuhara (1986)
Gadiformes									
Gadus morhua	3–4	3–6	17–18	2–3			60–90	5 & 10	Hunt von Herbing et al. (1996)

to date, the digestive tract differentiates into a stomach with gastric glands and pyloric caeca immediately prior to, or during metamorphosis (e.g. *Limanda yokohama*, Fukuhara 1988). In *A. melanopus* these organs had differentiated mid-way through the larva period.

When development is standardised by the time after fertilisation the relative rate of development of *A. melanopus* is faster than other tropical fish species. When egg development is included in age, *Amphiprion melanopus* are 16 days old at settlement, and have a full compliment of adult features. In comparison, 6 tropical Perciformes have total development ages at settlement ranging from 17 to 31 days (Job & Bellwood 2000).

Furthermore, the yellowtail damselfish, *Microspathodon chrysurus*, also a member of the Pomacentridae, does not undergo flexion until 16 days after hatching (Potthoff et al. 1987). In temperate fish such as *Gadus morhua* (Gadiformes) (Hunt von Herbing et al. 1996b), the embryos still have a yolksac until day 17 after hatching, while for *Hippoglossus hippoglossus* (Blaxter et al. 1983) and *Limanda yokohama* (Fukuhara 1988) (Pleuronectiformes) the gut has not even differentiated until 40 and 60 days after hatching, respectively (Table 3). Whilst phylogenetic differences confound a true comparison between Perciformes and other orders, and temperature differences confound a comparison between temperate and tropical species, it is still interesting to consider the style that this tropical Perciforme has adopted to reduce time until external feeding competency and therefore increase their chance of survival.

Implications of fast development

A comparison between *A. melanopus* and other tropical reef fish shows clearly that relative to other species *A. melanopus* is one of the fastest developing reef fish studied to date (Fisher et al. 2000, Job & Bellwood 2000). Within days of hatching *A. melanopus* are capable of substantial critical and sustained swimming (Fisher et al. 2000). Further for any given age *Amphiprion melanopus*, and the closely related *Premnas biaculeatus*, have the greatest functional visual sensitivity of 7 species studied (Job & Bellwood 2000). The extended duration in the egg envelopes and the relatively brief larva period, combined with extensive development within the first days after hatching would increase their potential to remain near their natal reef (Munday & Jones 1998). Self-seeding recruitment is documented for another pomacentrid species (Jones et al. 1999), and based on genetic differences within local populations limited dispersal has been proposed in 2 species of *Amphiprion* (Bell et al. 1982, Doherty et al. 1995). This may be an important adaptation to a patchy and highly specialised habitat, and perhaps a life history style to take some of the lottery out of recruitment success (see Sale 1978).

Critical periods?

The extensive development that occurred at the transition from endogenous nutrition to exogenous feeding suggests that this is a critical period in the development of *A. melanopus*. The alimentary canal and the stomach differentiate, and the jaw and related feeding structures calcify. Yolksac absorption is the period of most intense and rapid morphological development.

The changes that occur around settlement are not as dramatic in *A. melanopus* as observed in some other reef fishes (e.g. McCormick 1993, Shand 1994) as they have already developed many of their adults characters. The major changes that occur around the time of settlement are the completion of calcification of the fins and full adult pigmentation. The fins aid manoeuvrability in and around reefs, and in particular within anemones, while colour aids in camouflage and recognition of conspecifics.

The early larva period of *A. melanopus* is a period of intense development where critical structures are forming and developing, followed by a period of systems refinement prior to settlement, rather than a second period of critical development. In general the rate at which development occurs suggests a life history style aiding the vulnerable pre-competent life stages to make it through critical periods in development and survive the pelagic environment.

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