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Comparative study of metamorphosis in tropical reef fishes

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Abstract This study explores the types of changes in pigmentation and morphology that occur immediately after settlement in 13 families of tropical reef fishes encompassing 34 species. The morphology of individual fishes was recorded daily from when they were first caught at night as they came into the vicinity of a reef to settle. Changes in pigmentation and morphology were species specific and often varied greatly among species within a family or genus. Pigmentation changes were typically rapid (< 36 h) and dramatic. Morphological changes involved the elongation and regression of fin spines and changes in head shape and body depth. Eighteen percent of species experienced changes in snout shape and dorsal spine length of greater than 5%. Similarly, 15% experienced changes in pectoral fin length and head length of greater than 5%. Changes typically occurred gradually over 6 or more days, although in about 44% of the species the major change in one of the measured body dimensions occurred rapidly (within 36 h). Moderately strong positive relationships were found between both growth and developmental rates and the extent of metamorphosis in the damselfishes (Pomacentridae) ($r = 0.48$ and 0.63, respectively). This suggests there may be a minimum level of development necessary to be a fully functional demersal juvenile. Although many of the changes that occur are subtle compared to the preceding development, these changes occur at an important ecological transition.

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Introduction

Demersal fishes have complex life cycles that involve an ontogenetic change in morphology, physiology, and behaviour as their pelagic larval stages colonize benthic habitats. The term 'metamorphosis' is used to encompass the changes in structure and function that occur as a fish takes on its juvenile form, which often coincides with settlement. Metamorphosis is thought to occur because individuals must possess characteristics that maximize survival in each environment (Werner 1988). Both vertebrate and invertebrate marine larvae are specialized for dispersal with features suited to acquiring energy for development from their pelagic environment, evading predators, and finding a suitable location for the second part of their life cycle. Demersal life stages have markedly different energy requirements, with an energy regime devoted to growth and reproduction, and are exposed to differing sensory stimuli and mortality agents.

There is a paucity of information on what characterizes metamorphosis for demersal fishes and the time scale on which it occurs. Larval collections have shown that the loss of larval characters and the development of the juvenile form can be gradual, but often it occurs abruptly, especially in fish that are demersal as adults (Leis and Carson-Ewart 2000). Because morphological changes at the end of the larval phase can be rapid, it is often unclear from these collections the extent to which these transformations coincide with the settlement to the demersal habitat.

Understanding the extent to which a fish undergoes structural reorganization and development after settlement enhances our understanding of the processes that will impinge on their ecology during this important transition period. For example, fish that are markedly different from the juvenile form when they settle (e.g.

dartfish, scarids, and some labrids) have an extended transition period after settlement before they join the main juvenile population (McCormick and Makey 1997). McCormick and Makey (1997) found that the dartfish, *Pteroleotris evides*, underwent a series of three identifiable transitions, involving different morphology and habitat associations, over a 3-week period before adopting a more stable juvenile morphology and ecology. In contrast, the damselfish, *Pomacentrus amboinensis*, which undergoes little structural change, settled directly into its adult habitat. It is probable that mortality schedules may be linked to the complexity of the morphological and ecological changes that occur soon after settlement.

Indeed, there has been surprisingly little research on the changes that occur at the end of the larval phase given that this life-history transition is referred to by a number of researchers as a 'critical period' (e.g. Blaxter 1988; Thorisson 1994). The lack of research is particularly puzzling given the emphasis on the importance of replenishment of populations through larval sources via settlement for populations of demersal fishes (Doherty 1991; Doherty and Fowler 1994; Caley et al. 1996). Researchers that emphasize the importance of metamorphosis typically cite literature on temperate flatfish (e.g. Fukuhara 1988; Evans and Fernald 1990; Markle et al. 1992; Keefe and Able 1993), or those fish with leptocephalous larvae, such as bonefish (Pfeiler 1986), tarpon (Tsukamoto and Okiyama 1997), eels (Arai et al. 1997), and lampreys (Youson 1988). There are few examples of the extent of restructuring that occurs at the end of the larval phase, and there is currently no information as to whether generalities can be made.

The objective of the present research was to explore the types of changes in pigmentation and morphology that occur during the settlement transition for 34 species from 13 families of tropical reef fishes. Specifically, the study addressed the following questions: (1) What are the morphological changes associated with settlement in a range of tropical reef fish families? (2) How does the degree of morphological change at settlement compare amongst fish species? (3) Is the magnitude of metamorphosis associated with larval growth or developmental rates?

Materials and methods

Fish collection

Fish larvae were collected at two localities in the tropical Pacific: Lizard Island on the northern Great Barrier Reef (14°41′S, 145°27′E) and Rangiroa Atoll in the Tuamotu Archipelago, Tahiti. At Lizard Island fish were caught using light traps (Stobutzki and Bellwood 1997) deployed around the island during November to December 1994 and 1997. Traps were moored over sand approximately 100 m from the edge of the fringing reef. Fish that were caught in the traps were removed during darkness, between 2300 and 0200 hours, placed in buckets of aerated seawater, and returned to the laboratory.

At Rangiroa Atoll fish larvae were collected on the reef crest at night using a plankton net (3.5 m wide, 1 m high, 5 m long of 1-mm mesh) fixed to the substratum (Dufour et al. 1996). This net was positioned in a shallow channel that connected the lagoon to the ocean. In this way larvae were passively collected by the net as water passed over the reef crest and flat to enter the lagoon. Fish were removed from the net between 2130 and 2230 hours and taken to the laboratory for sorting and processing (see below).

Quantification of morphology and pigmentation

Metamorphosis was quantified by using a Hi-8 video camera to record images of individual fishes over 4–10 days post capture. Upon capture and sorting in the laboratory, selected fish were anesthetized with MS222 and placed into a small, narrow aquarium (3×15×12 cm). Fish were carefully restrained against the front of the aquarium by an extra piece of glass and a series of video images were captured against a scale bar. These images could then be digitized using a computer image-grabber (miroVIDEO DC30+ video capture board; Pinnacle Systems, Inc.) and imported into an image analysis package (Image Tools) to quantify various morphological variables. Fish were kept in individual tanks and fed 24- to 36-h-old *Artemia* sp. nauplii, and video images were captured once a day. Acanthurids were also supplied algal-covered coral rubble as a feeding substratum. Initial recordings of body morphology were made within 2 h of capture.

Eleven morphological variables were recorded from the video images. The seven found most useful for statistical discrimination of species are shown in Fig. 1. These variables were (1) standard

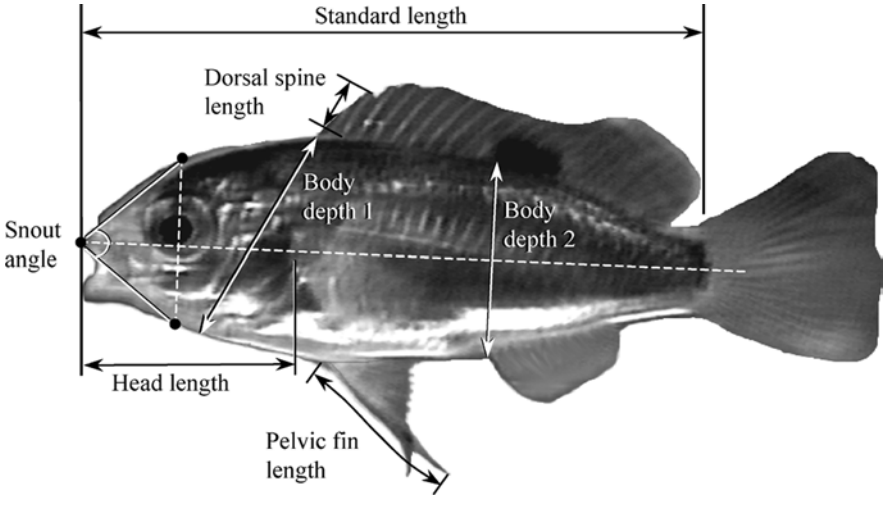

Fig. 1 *Chrysiptera leucopoma*. Seven morphological measurements made on captured video images of live fish to assess morphological change during metamorphosis. These measurements were those used in statistical analyses of morphological change

length; (2) head length; (3) pectoral fin length; (4) length of the first or second dorsal spine (whichever was longest); (5) depth of the body from the first dorsal spine to midway between the base of the pelvic fin and anterior-most part of the dentary (body depth 1); (6) depth of the body from the vent to the base of the dorsal fin, perpendicular to the main axis of the body; (7) angle of the snout, measured as the angle of intersection of two lines to the anterior-most part of the snout. The start of these two lines are defined as where a line that is perpendicular to the main axis of the body and that passes through the mid-point of the eye bisects the ventral and dorsal head (see Fig. 1). Additional variables were total body length, anal spine length, eye diameter, and maximum body depth (perpendicular to the main axis of the body). Snout angle was measured three times for each fish and the average angle was used in analyses. To examine the change in body dimensions over time the linear body measurements were expressed as a proportion of the standard length. While video images of the fish were being recorded detailed notes on the degree of transparency and patterns of pigmentation were collected.

Initial trials to obtain reliable estimates of morphology from the videotapes indicated that a strict protocol was necessary. Only videotape frames in which fish were against and parallel to the front glass of the aquarium, and had their mouths closed, were captured. In addition, measurements were calibrated using as much of the scale bar as possible to minimize error. To determine the precision of quantifying morphology from live specimens using the video technique described, a hierarchically designed pilot study was undertaken. This examined the magnitude of the errors associated with each step of the image capture and measurement process. Video footage of five *Acanthurus triostegus* when first caught in the crest nets were chosen; from each of the five videos five frames were grabbed for measurement; each frame was calibrated three times, with each of 11 morphological variables measured on each frame three times (i.e. each fish×frame×calibration combination was measured three times, totaling 225 images).

Measurements were undertaken on 34 species from 13 families (Table 1). Due to the limited availability of individuals of each species, morphological measurements were only made on a small number of individual for each species ($n = 1$–4; mean 2.6). The mean proportional change (described below) of the replicate fish for each species was used to characterize each species. The morphological changes associated with settlement are regarded as developmental characteristics and as such are likely to demonstrate little variability in the nature of change. The temporal scale on which these changes occur could be expected to differ among individuals but this is anticipated to be relatively minor compared to the differences among species. All individuals used in the study associated with the bottom of the holding tanks the morning after capture, suggesting that they had adopted the demersal phase of their life history. As an initial part of the analysis of data, the amount of variability in morphological change over metamorphosis within a species was examined to assess the validity of using changes in one fish to characterize morphological change in a species.

It should be noted that some studies have suggested that artificial conditions within an aquarium or laboratory may alter the pace of changes that occur at settlement (Randall 1961; Grover et al. 1998). Although this may have affected the rates of change in the current study, it is not thought to influence the types or magnitudes of changes that are assessed here for comparative purposes.

Metamorphosis, growth, and developmental rate

The first two components of a principal component analysis (PCA; see analysis section below for details) can be used as summaries of the relative extent of morphological change among species over the 3.5–4-day period used in calculations. The relationship between these indices of metamorphosis and average larval growth and developmental rate was examined. Pelagic larval durations (PLDs) of damselfishes were obtained from Wellington and Victor (1989) for all species except *Pomacentrus amboinensis* and *P. nagasakiensis*, where large samples of light-trap-caught fish were available. Otoliths of these two species were processed using the methodology of Wilson and McCormick (1999). Growth rate (millimetres per day) and developmental rate (1/PLD) were then calculated. Due to the lack of availability of larval durations for all species of fish (some samples were misplaced, and many were not available from the literature), analysis is restricted to the Pomacentridae. This also reduces phylogenetic confounding in any evolutionary interpretation.

Analysis

To allow a comparison among species of the relative magnitude of morphological change at settlement an interval of 3.5–4 days was chosen for analysis between first record of morphology (i.e. the image taken immediately upon capture) and end-point. To avoid the confounding influence of differing fish size, the proportional change in each dimension was calculated and used in analysis:

$$\%\ \text{change Var1} = [(\text{Var1}_{t2}/\text{SL}_{t2}) - (\text{Var1}_{t1}/\text{SL}_{t1})] \times 100$$

where SL_{t1} is the standard length when first videotaped (t1), SL_{t2} is the standard length 3.5–4 days after capture (t2), Var1_{t1} is the length of a particular body dimension when first videotaped, and Var1_{t2} is the length of the same body dimension 3.5–4 days after capture. Values were either positive or negative, representing an increase or decrease in that particular body dimension with time, respectively. No change was indicated by values close to zero. The one exception to this standardization was for the variable 'snout angle' (Fig. 1), which was represented simply as the difference between the angle at 3.5–4 days post capture and angle obtained from the initial video image.

The assumption that one fish could characterize the changes in morphology that occur around settlement was assessed using cluster analyses undertaken on the proportional changes of six species for which replicate fish had been measured. The six species used in these analyses, spanning a range of body morphologies, were *A. triostegus*, *Chromis viridus*, *Chrysiptera leucopoma*, *Lethrinus* sp., *P. nagasakiensis*, and *Diploprion bifasciatum*. The variable 'total length' was dropped because of its high correlation with SL. The number of variables was further reduced by dropping those variables that scored highest on the last principal component of preliminary PCAs, leaving in the model the variables body depth 1, body depth 2, head length, pelvic fin length, dorsal spine length, and snout angle. Both average linkage and Ward's hierarchical cluster analyses (SAS Institute Inc. 1987) on Euclidean distances were undertaken to check the consistency of clusters found.

PCA on the covariance matrix was used to explore the changes in morphology that occur around the time of settlement and how they differ among the 34 species collected. The PCA was interpreted in relation to the original body variables by plotting the eigenvalues as eigenvectors from the origin (Euclidean distance biplot). The relative length and direction of these vectors indicates the direction and importance of trends in the original variables. Average linkage and Ward's hierarchical cluster analyses were then undertaken on the same data set and the results superimposed on a plot of the first three components of the preceding PCA. The number of clusters was determined by a peak in the values of the cubic clustering criterion (SAS Institute Inc. 1987).

Results

Methods verification

The overall errors associated with obtaining measurements from the videotapes were very low [e.g. coefficient of variation (CV) values: SL, 1.1%; body depth 2, 2.4%; head angle, 1.5%; Table 2]. The small magnitude of these errors suggests that morphology can be reliably quantified from videotapes, when a strict data collection

Table 1 Change in morphology of 34 species of tropical reef fishes from capture to 3.5–4 days afterwards. The mean size at capture (standard length, *SL*, in millimetres) of each species is given together with the cluster to which they were assigned, based on changes in morphology measured over 3.5–4 days after capture. To summarize body changes over this period the change in the body proportions (expressed as a percentage of SL) is given for five morphological variables (defined in the text). *Plus signs* represent an elongation of the attribute; *negative signs* represent regression. Changes of less than 1% are listed as zero values. Values greater than 5% are highlighted in *bold*. Species identification (ID) numbers used in Fig. 4 are listed. *Diploprion bifasciatum* was excluded from the analysis presented here because of the high magnitude of change in its dorsal spine swamped all other trends among species. *Asterisks* represents species collected from Tahiti

Family	Species	Species ID	Cluster	Size (SL)	Percentage change in morphology				
					Snout	Dorsal spine	Pectoral fin	Max. body depth	Head length
Acanthuridae	*Acanthurus triostegus**	1	E	24.6	–2.3	–1.0	+1.0	**–5.8**	–2.5
	A. xanthopterus	2	E	33.3	–0.9	0	**+5.1**	**–7.3**	**–6.2**
Apogonidae	*Apogon* sp. 1	3	F	10.2	0	0	0	–3.0	–1.5
	Apogon sp. 2	4	F	22.2	–2.5	–1.1	–4.2	0	+4.2
Balistidae	*Rhinecanthus aculeatus**	5	F	22.8	–2.1	0	0	0	0
Caesionidae	*Caesio cunning*	6	C	20.5	**+13.0**	**–9.8**	–1.1	0	+1.2
Chaetodontidae	*Chaetodon ephippium*	7	F	15.6	0	0	0	–1.6	–1.2
	*C. lunula**	8	F	13.3	0	0	0	–2.2	–1.5
	C. plebeius	9	F	10.4	–3.5	0	–3.9	–4.6	+1.2
	Chelmon rostratus	10	D	16.1	**–6.9**	**–5.5**	**+5.6**	+2.3	0
Holocentridae	*Myripristis kuntee**	11	F	43.7	0	+1.1	+1.3	–1.7	+1.3
	Myripristis sp. 1*	12	F	47.1	0	0	+2.3	0	+1.3
	Myripristis sp. 2*	13	F	33.0	+4.7	–1.8	–3.6	+1.2	0
	*Sargocentron rubrum**	14	A	29.4	**+16.6**	0	+3.9	+2.2	0
Lethrinidae	*Lethrinus atkinsoni*	15	D	20.5	**–5.5**	–1.4	+2.9	+2.3	**+8.5**
	L. genivittatus	16	B	18.2	+2.8	**+6.2**	+2.9	+2.1	+1.1
	Lethrinus sp.	17	F	17.5	+2.8	0	+1.8	+3.1	0
Monocanthidae	*Oxymonocanthus longirostris*	18	F	21.4	–1.4	0	–2.1	0	+1.5
	Monocanthid sp. 1	19	F	23.1	–4.6	0	–2.5	–3.9	0
Mullidae	*Parupeneus multifasciatus*	20	B	66.7	+3.9	**+5.5**	+3.5	0	0
Pomacentridae	*Chromis viridus**	21	F	8.1	+3.5	–1.5	–1.2	+1.5	**+5.1**
	*Chrysiptera leucopoma**	22	F	15.7	+2.2	+2.1	–1.2	0	0
	*Plectroglyphidodon lacrymatus**	23	F	10.2	0	–4.5	–2.3	–2.2	+4.6
	Pomacentrus amboinensis	24	B	11.5	+3.6	+2.2	**+5.9**	+3.1	0
	P. bankanensis	25	A	14.8	**+9.4**	+3.5	+2.7	+1.5	–3.0
	P. moluccensis	26	F	11.3	–1.5	–2.4	–1.5	+2.0	+2.7
	P. nagasakiensis	27	B	13.0	+4.0	+2.3	+4.7	+2.5	0
	*P. pavo**	28	F	15.5	+3.3	0	0	0	–1.2
	*Stegastes nigricans**	29	A	13.1	**+10.1**	0	0	**+11.7**	**–5.7**
Scorpaenidae	Scorpaenid sp.	30	B	9.0	+4.8	0	**+6.3**	+1.5	**+6.6**
Serranidae	*Gramistes sexlineatus**	31	F	10.8	–1.6	+2.7	0	–1.8	+2.7
	Plectropomus leopardus	32	C	19.5	–2.2	**–9.7**	**–13.0**	0	0
	Diploprion bifasciatum	–	–	24.9	–3.3	**–111.0**	–4.0	–2.0	+2.3
Tetradontidae	*Ostracion cubicus**	33	E	11.4	+4.2	–4.4	0	–4.2	–1.3
Percentage of species that showed >5% change in body dimensions					17.6	17.6	14.7	8.8	14.7

Table 2 *Acanthurus triostegus*. Examples of the percentage of total variance associated with each stage of making morphological measurements from images captured from videotapes. Video footage of five different *A. triostegus* when first caught were chosen. From each video five frames were grabbed for measurement; each frame was calibrated three times, and measures were repeated three times. The coefficient of variation (*CV*) of all sources of error (i.e. the factor *Frame* and below) is also given (%), as an indicator of the actual magnitude of measurement error

Variable	Fish (5)	Frame (5)	Calibration (3)	Replicates (3)	CV
Standard length	88.5	4.8	3.9	2.8	1.1
Body depth 2	91.7	6.0	1.1	1.2	2.4
Head angle	89.0	8.3	0.0	2.7	1.5

protocol is used. Most of the variability was explained by differences in size among fishes (>88%), with differences among frames within a video sequence being the greatest source of measurement error accounting for 5–8% of the total variability in measurements (Table 2).

Analyses suggest that one fish can be used to characterize the changes in morphology that occur around settlement in that species. Average linkage and Ward's cluster analyses produced exactly the same clusters of the six species for which replicate fish had been measured. Replicate individuals within a species tightly clustered together, with no overlap among species (Fig. 2). This suggested that the morphological changes at settlement in a single fish may be used to characterize morphological changes that occur in the species.

Changes in morphology

The 34 species of fishes displayed a diversity of morphological changes over the 3.5- to 4-day interval chosen for the among-species comparisons. Both Average Linkage and Ward's cluster analyses found two consistent groups of species based on the types of morphological changes. The serranid *Diploprion bifasciatum* was the only species in one of these groups. This species was unique in possessing long fleshy banners from the second and third dorsal spines, which were lost within 24 h of settling (Fig. 3a, b). The magnitude of this change (for one individual, 46.7 mm to 5.7 mm in 3 days for the second dorsal spine) overwhelmed the more subtle changes that occurred in the other 33 species. For this reason a PCA was run twice, once with *D. bifasciatum* and once without to explore the differences among the remaining species with greater resolution.

When the PCA and cluster analyses were re-run without *D. bifasciatum*, six groups were consistently identified (groups A–F, Fig. 4, Table 1). Cluster analyses showed that the main differences in the types of morphological change were between the 8 species from groups A and B (Fig. 4a, Table 1), and the remaining 25 species. Group C is the next most pronounced division, followed by group D. Lastly, the 3 species in group E split from the remaining 18 species (group F). Groups separated from one another along the first three axes of variability in the data set (PCs 1–3), together explain 76% of the total variability in the data set (Fig. 4).

Changes in snout angle (PC 1) and dorsal spine length (PC 2) were the main trends responsible for the major split between groups A and B and the other four groups. Fishes in groups A and B were characterized by increases in snout angle and dorsal spine length, relative to the other species (Fig. 4, Table 1). The 2 species in group C (*Caesio cunning* and *Plectropomus leopardus*) exhibited marked reductions in dorsal and/or pectoral fin spines of up to 13% (Table 1). On the other hand, the 2 species in group D (*Lethrinus atkinsoni* and *Chelmon rostratus*) showed reductions in snout angle of about 6% and increases in both relative pectoral fin length and body depth (Table 1). In contrast, the 3 species in group E (*Acanthurus triostegus*, *A. xanthopterus*, and *Ostracion cubicus*) showed decreases in body depth and head length of up to 7% (Table 1). Lastly, there was a large group of 18 species (group F) that were characterized by moderately low levels of change in body morphology over the 3.5- to 4-day post-settlement period (Table 1).

Interestingly, the members of the Pomacentridae, represented by 9 species, were present on both sides of the major split in the trends in morphological change (Fig. 4, Table 1). Two of the 9 species experienced marked increases in the angle of the snout (9–10%), 5

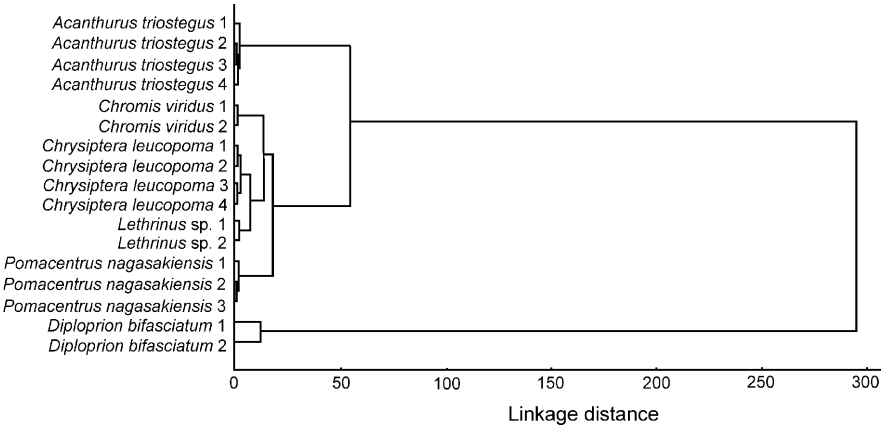

Fig. 2 *Acanthurus triostegus*, *Chromis viridus*, *Chrysiptera leucopoma*, *Diploprion bifasciatum*, *Lethrinus* sp., *Pomacentrus nagasakiensis*. A comparison of the morphological changes from capture to 3.5–4 days post settlement among six species of reef fishes for which replicate individuals were obtained. The dendrogram is the results of a Ward's cluster analysis on Euclidean distances

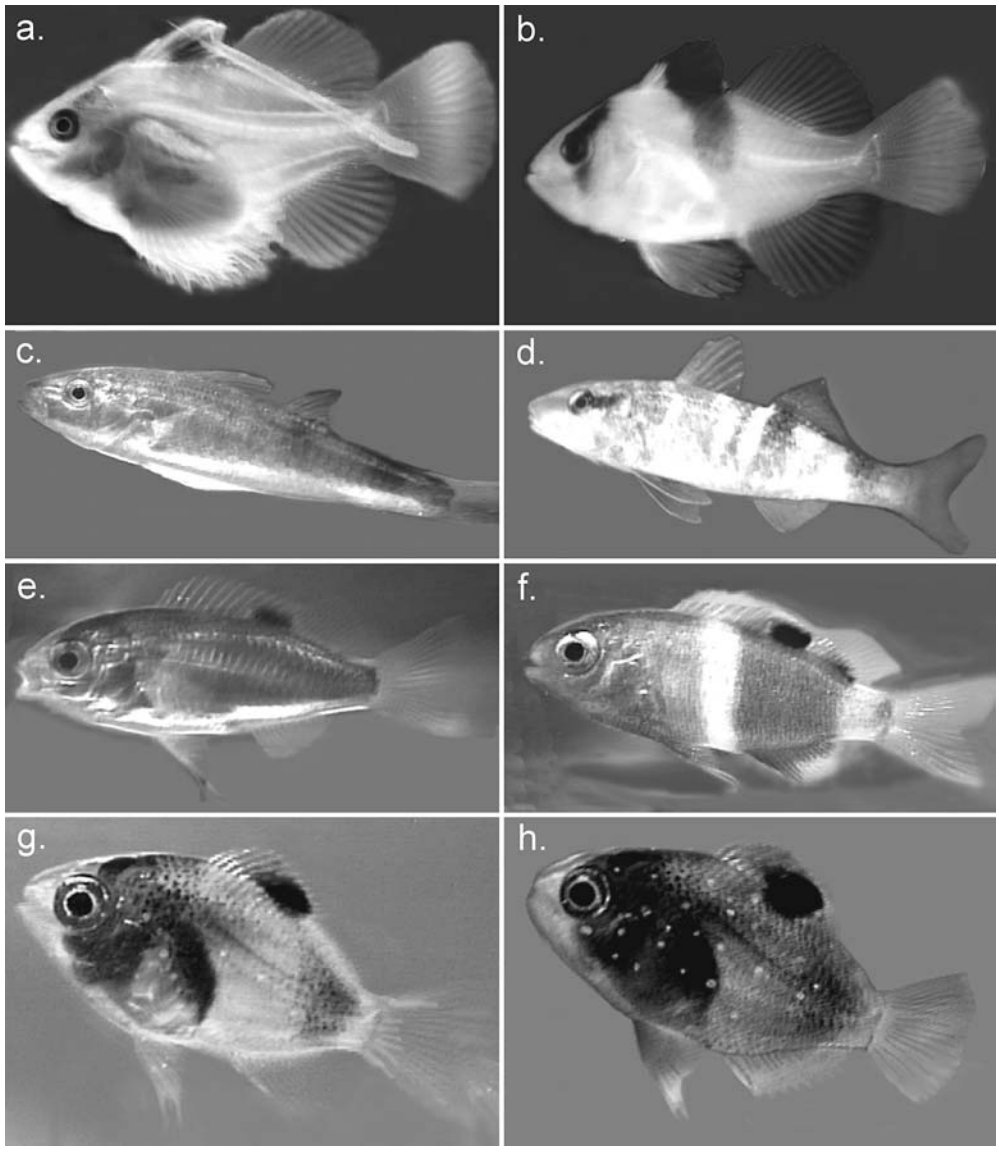

Fig. 3a–h *D. bifasciatum*, *Parupeneus multifasciatus*, *C. leucopoma*, *Plectroglyphidodon lacrymatus*. A comparison of pigmentation for four species of coral reef fishes at the end of the larval phase (**a**, **c**, **e**, **g**) and 3.5–4 days after settlement (**b**, **d**, **f**, **h**). The species are (**a**, **b**) the serranid *D. bifasciatum*; (**c**, **d**) the mullid *Parupeneus multifasciatus*; (**e**, **f**) the pomacentrid *C. leucopoma*; (**g**, **h**) the pomacentrid *Plectroglyphidodon lacrymatus*

experienced moderate increases, whilst the remaining 2 species showed no change or a small negative change. Similarly, 3 species experienced increases in head length, 2 experienced decreases, with the remainder displaying little or no change (Table 1). The 4 species examined within the Chaetodontidae showed a high level of variability in morphological change similar to that shown within the Pomacentridae. *Chelmon rostratus* underwent marked reductions in snout angle (6.9%) and dorsal spine length (5.5%), combined with an increase in pectoral fin length (5.6%), which were changes not found in the 3 *Chaetodon* species (Table 1). Even within the genus *Chaetodon*, *C. plebeius* showed very different changes in morphology over the first 3.5–4 days from those shown by *C. lunula* or *C. ephippium*. Indeed, most families examined that had replicate species showed high levels of variability in magnitude and direction (increase or decrease) of morphological changes (Table 1).

Six species showed a proportional decrease in standard length over the first 4 days after capture of greater than 5% of their original body length. These decreases in length occurred despite being fed ad libitum. Feeding in all fish was observed during the maintenance period. It is possible that the food given was either unsuitable for growth, particularly for the herbivores (although these were also given access to algal-covered rubble). Two acanthurids (*A. triostegus*, *A. xanthopterus*), two pomacentrids (*Chrysiptera leucopoma*, *Pomacentrus nagasakiensis*), an apogonid (Apogonid sp. 2) and a holocentrid (*Sargocentron rubrum*) displayed shrinkage over the first 4 days after capture.

The patterns of change over the first 6 days after capture suggested that whether the change in a particular body variable was gradual or rapid differed among taxa, although a gradual change appeared to be more common (Fig. 5). The limited data available suggest that the angle of the snout generally changes gradually (Fig. 5a). One exception may be for *P. bankanensis* where there was a 6° increase in snout angle over the first day. Three species (*Caesio cunning*, *D. bifasciatum* and

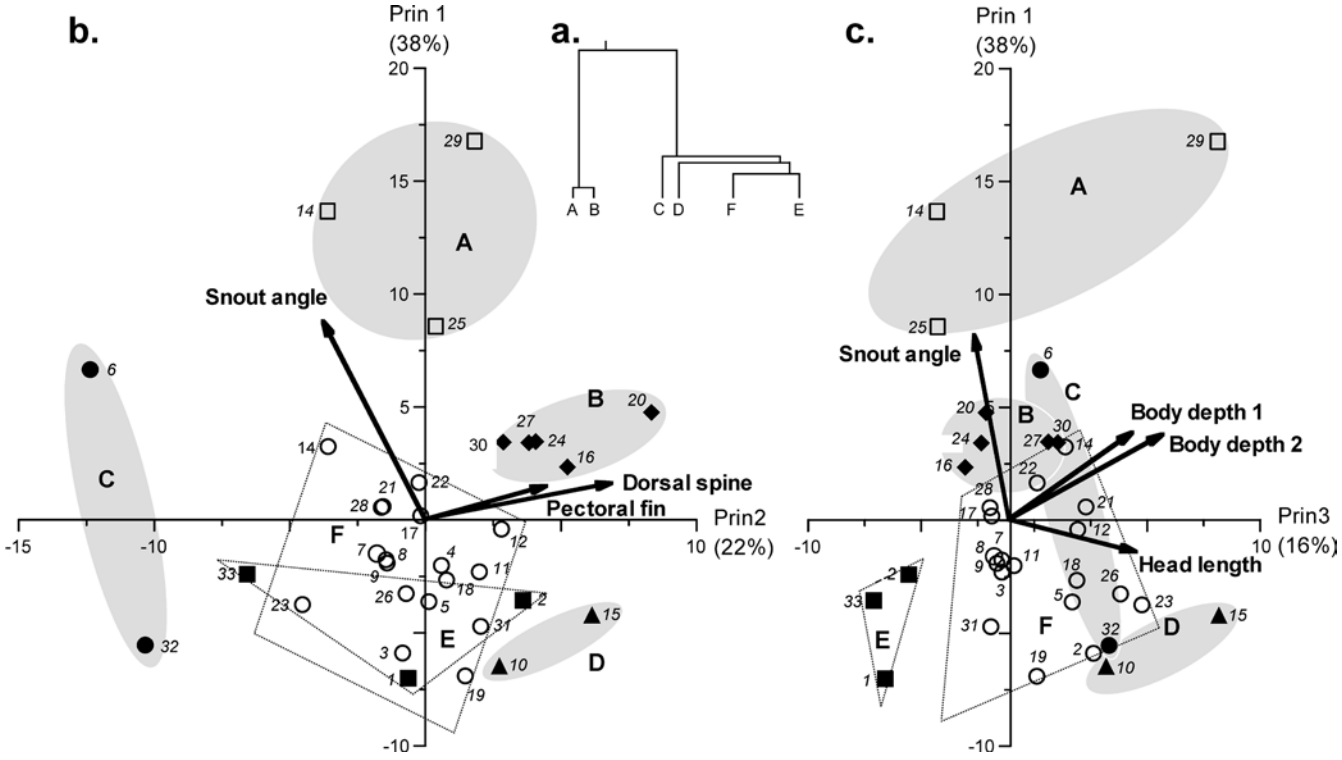

Fig. 4a–c Comparison of morphological change from capture to 3.5–4 days post settlement among 33 species of tropical reef fish. A cluster analysis shows the relationship among clusters (**a**) and these clusters have been superimposed on a principal component analysis that displays the pattern of variability in body morphology (expressed by seven variables shown in Fig. 1) among fish species. Species in the same cluster are displayed by the same *symbol*. The *vectors* show the trends in the original body variables that explain the main differences among species. *Numbers* represent species and are listed in Table 1. Plots between (**b**) principal components 1 (*Prin1*) and 2 (*Prin2*) and (**c**) principal components 1 and 3 (*Prin3*) are given

Chelmon rostratus) showed a marked reduction in the length of their dorsal spines. In two of these species the changes were dramatic and rapid, with *D. bifasciatum* showing a 110% reduction of spine length relative to SL in the first day, whilst *C. cunning* experienced a 7% reduction. Although there was a 7% reduction in relative spine length in *C. rostratum* over its 6-day sampling period, this change occurred gradually (Fig. 5b). Most species experienced gradual changes in the proportion of the head length to body length, although a rapid 7% reduction during the first day was shown by *A. xanthopterus* (Fig. 5c). Even though data are limited, there appears to be an indication in most species that the degree of change in body proportions is slowing down by 5–6 days after capture (Fig. 5).

Changes in pigmentation

Detailed descriptions were recorded on changes in pigmentation with settlement (Table 3). When first examined the night they were caught, all but 2 species had either transparent, silver, or bronze muscle tissue and most had transparent fins (see Fig. 2 for examples). Three out of four members of the family Holocentridae had bright markings on the anterior part of their dorsal fin. In contrast, the two late-larval specimens of the serranid *D. bifasciatum* (Fig. 2a, b), although transparent, were both bright yellow. Similarly, all specimens caught of the boxfish *O. cubicus* (more than ten individuals from crest nets) had a bright yellow cube-shaped non-transparent interior surrounded by transparent outer tissue.

Fourteen out of the 34 species examined took less than 36 h to attain their initial benthic-stage pigmentation. This involved a loss of body tissue transparency for all but the apogonids. Fishes that showed rapid changes in pigmentation were the chaetodontids, holocentrids, monocanthids, mullids, pomacentrids of the genera *Chyrisiptera* and *Pomacentrus*, and tetradontids. Some changes to the pelagic pigmentation occurred immediately in all species, with the exception of the pomacentrid *Chromis viridus*, which showed no change over the 5-day observation period. For some species pigment changes were relatively slow. The 3 lethrinid species took 2.5–3 days to lose transparency of body tissues and take on a cream or light green hue. After 7 days of observation the scorpaenid species had only acquired pigmentation on the anterior two-thirds of its body. The pomacentrids *Plectroglyphidodon lacrymatus* (Fig. 2g, h) and *Stegastes nigricans* were also slow in attaining benthic colouration, taking 3 and 7 days, respectively.

A sequence of colour changes occurred in the coral trout *Plectropomus leopardus*. Within 2 h of capture fish

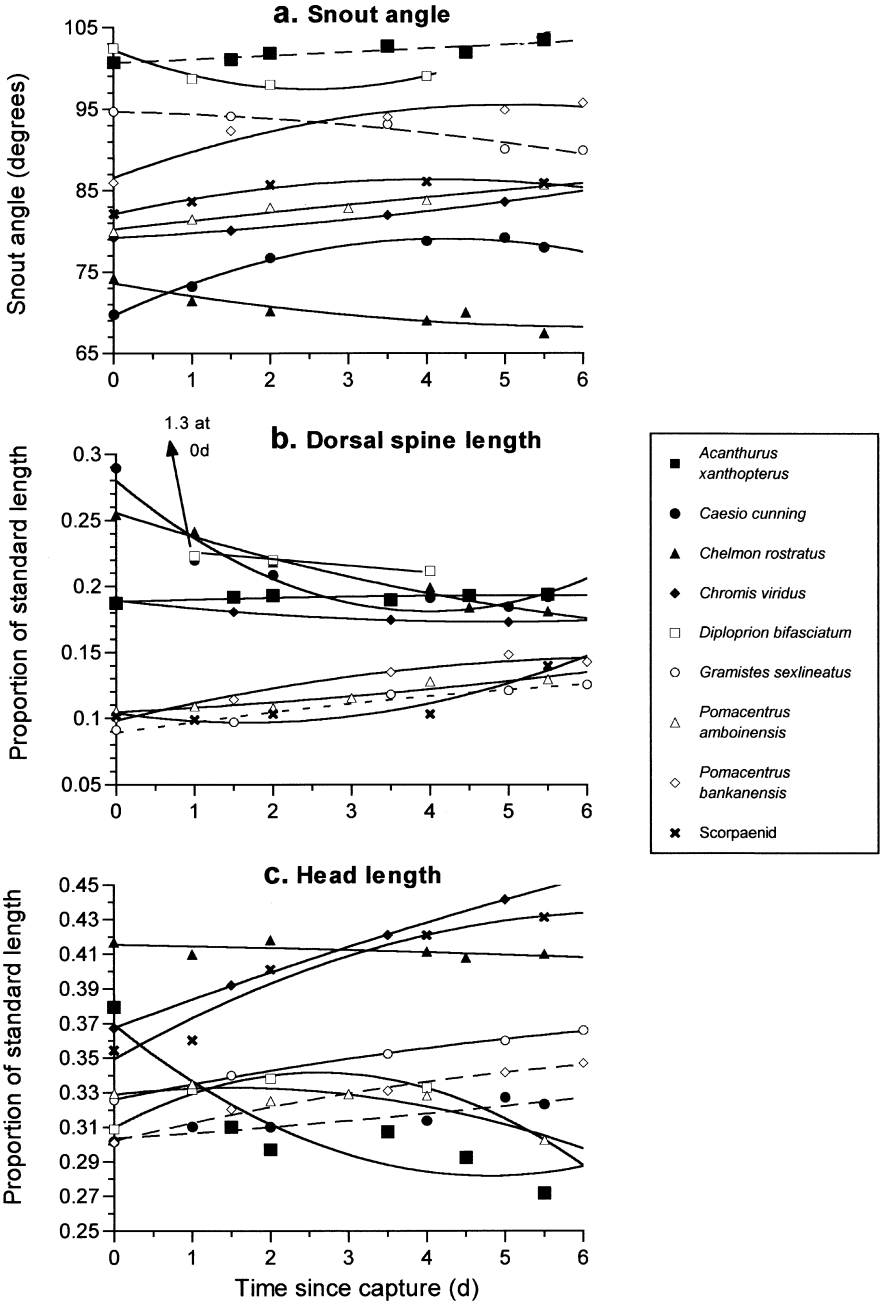

Fig. 5a–c Changes in the size of three body attributes for nine species of fish over the first 6 days after capture. (**a**) Snout angle (degrees); (**b**) length of the larger of the first or second dorsal spines expressed as a proportion of standard length; (**c**) length of the head expressed as a proportion of standard length. *Curves* fitted to species are simply to smooth trends rather than describe relationships (all are quadratic, with the exception of snout angle for *D. bifasciatum*)

changed from transparent to bright red/orange. A white horizontal stripe developed along the spine after 2 days, and by 4 days this white stripe was bound by disrupted black stripes, the lower stripe of which passed through the eye. Eleven days later the black stripes had concentrated into small discrete spots. The unidentified scorpaenid took the longest to change to a reef colouration; in the first 4 days, only the head and anterior of the body darkened and developed a mottled pattern. It was not until 6 days later that faint stripes started to occur on the posterior half of the body. After 10 days of observation, the posterior body still remained largely transparent, even though the fish sat on the bottom of the tank for the entire 10-day period.

Metamorphosis, growth, and developmental rate

To examine the relationships between the extent of metamorphosis, growth, and development among the nine species of pomacentrid included in the study, the first two PCs were used as summaries of the relative extent of morphological change among species. *S. nigricans* appears to be an outlier in the relationships between PC 1 and developmental rate or growth rate (Fig. 6a, b). Similarly, the species is also an outlier in the relationship between PC 2 and developmental rate (Fig. 6c). Once *S. nigricans* was excluded, there was a weak positive relationship between PC 1 (largely representing change in snout angle, see Fig. 2) and develop-

Table 3 Initial pigmentation of newly caught late-stage larvae and pigmentation of same individuals 4 days later. Estimated time for the main changes in pigmentation to occur is also given

Family	Species	Pelagic	4-day benthic	Time for main pigment change
Acanthuridae	*Acanthurus triostegus*	Transparent, silver gut and head	Cream, six vertical black stripes	36 h
	A. xanthopterus	Transparent, silver gut and head	Olive brown body, mustard dorsal and caudal fin, with three yellow horizontal stripes on anal fin	48 h
Apogonidae	*Apogon* sp. 1 (*augustatus*?)	Bronze body, clear fins	Silver, four thin black horizontal stripes	84 h
	Apogon sp. 2	Transparent, silver gut and head; pigment spots on anterior gut and head	Anterior half body pigment spots, posterior transparent	288 h[a]
Balistidae	*Rhineacanthus aculeatus*	Silver	Black/brown stripes on silver	>96 h
Caesionidae	*Caesio cunning*	Transparent body; pink gut; yellow peduncle spot	Silver-grey body; yellow from peduncle extending to base of dorsal fin; pink along backbone	84 h
Chaetodontidae	*Chaetodon lunula*	Gold/bronze body, clear fins	Gold body; black peduncle spot; black patch over eye; white behind eye to forehead; black patch behind headband; black ocellus on posterior dorsal fin	<8 h
	C. ephipium	Silver/white body; faint black patch on rear upper corner	Silver/white body; black patch on rear upper corner; brown vertical stripe through eye; yellow snout, breast, anal and posterior dorsal fins	<8 h
	C. plebius	Bronze body, clear fins	Yellow body; black vertical eye stripe; black spot on peduncle	<6 h
	Chelmon rostratus	Transparent with silver gut, clear fins	Silver/white body, four vertical brown stripes; black stripe on peduncle; black ocellus on dorsal	<12 h
Holocentridae	*Myripristis kuntee*	Silver, bronze on dorsal surface; clear fins	Red upper half body, plus fins except pelvic; silver lower body; white leading edge to 2nd dorsal, anal and pelvic fins	24 h
	Myripristis sp. 1	Silver, bronze on dorsal surface; white leading edge to 2nd dorsal, anal and pelvic fins; large black spot on pelvic fins; white on front of spines on 1st dorsal fin	Red body; white leading edge to 2nd dorsal, caudal, anal and pelvic fins; large black spot on pelvic fins; outer third of 2nd dorsal and anal fins dark red; yellow 1st dorsal fin	34 h
	Myripristis sp. 2	Silver body, dark dorsal surface; clear fins, except 1st dorsal, which is white with two large black patches	Upper third of body and ventral red-bronze, rest silver; fins clear except 1st dorsal, which is black with a large white patch in middle	<11 h
	Sargocentron rubrum	Silver-bronze body; clear fins except 1st dorsal, which is black with white spine tips, and large yellow patch in middle	Body dark red with white horizontal stripes; fins pink except 1st dorsal, which is black with white spine tips and large pink patch in middle	24 h
Lethrinidae	*Lethrinus atkinsoni*	Semi-transparent body, faint green head, faint white speckles over body; silver gut	Cream body	72 h
	L. genivittatus	Transparent, silver gut; faint silver/green on head	Cream body, green patches	72 h

Table 3 (Contd.)

Family	Species	Pelagic	4-day benthic	Time for main pigment change
	Lethrinus sp.	Transparent, silver gut; faint silver/green on head; pigment spots on head and top of swim bladder; faint brown body patches	Light green body; faint brown disrupted stripes	60 h
Monocanthidae	*Oxymonocanthus longirostris*	Silver body, green patches; six faint vertical stripes; black spot on pelvic fin and caudal base; brown dorsal spine	Aqua blue body; five thick disrupted horizontal stripes; five faint vertical black stripes	6 h
	Monocanthid sp. 1	Silver-cream body	Silver cream body, four faint yellow horizontal stripes on dorsal half of body	–
Mullidae	*Parupeneus multifasciatus*	Silver body, clear fins	Brown anterior, black stripes through eye; posterior cream with two black stripes	< 8 h
Pomacentridae	*Chromis viridus*	Bronze body, clear fins	No change	–
	Chrysiptera leucopoma	Silver, clear fins	Dark brown body, mid-body vertical yellow stripe; white stripe on caudal peduncle; yellow chest	< 6 h
	Plectroglyphidodon lacrymatus	Transparent body, silver gut	Brown body, blue spots, large black ocellus on anterior dorsal fin	72 h
	Pomacentrus amboinensis	Transparent body, silver gut and head; pigment spots along posterior margins of body and spine; black ocellus on dorsal fin	Yellow body; white ring around black dorsal ocellus	< 6 h
	P. bankanensis	As for *P. amboinensis*	Dark grey body; large dorsal ocellus; white caudal base; blue lines dorsally on head	12 h
	P. moluccensis	Transparent body, silver gut and head	Yellow body and fins	< 6 h
	P. nagasakiensis	As for *P. amboinensis*	Blue body; white ring around black dorsal ocellus	< 6 h
	P. pavo	Transparent body, silver gut and head	Bright blue body; yellow caudal fin, peduncle, anal and posterior dorsal fin	32 h
	Stegastes nigricans	Silver body; transparent snout	Same except black spot on anterior of dorsal fin	168 h
Scorpaenidae	Scorpaenid sp.	Transparent, pink gut	Posterior half transparent; anterior brown with cream blotched	> 168 h
Serranidae	*Gramistes sexlineatus*	Transparent, yellow over silver gut	Black with white spots; spot on gut yellow; clear fins	96 h
	Plectropomus leopardus	Transparent, with faint orange on head	Pink/red with one white horizontal stripe along spine, black diffuse horizontal stripes above and below white stripe	60 h
	Diploprion bifasciatum	Transparent bright yellow; pink gills and gut	Yellow body; black stripe through eye; thick black stripe from 1st dorsal to mid-lateral	< 24 h
Tetradontidae	*Ostracion cubicus*	Yellow interior with transparent tissue outer	Yellow body, black spots	6 h

[a] Pigment spots to base of peduncle, but body still transparent

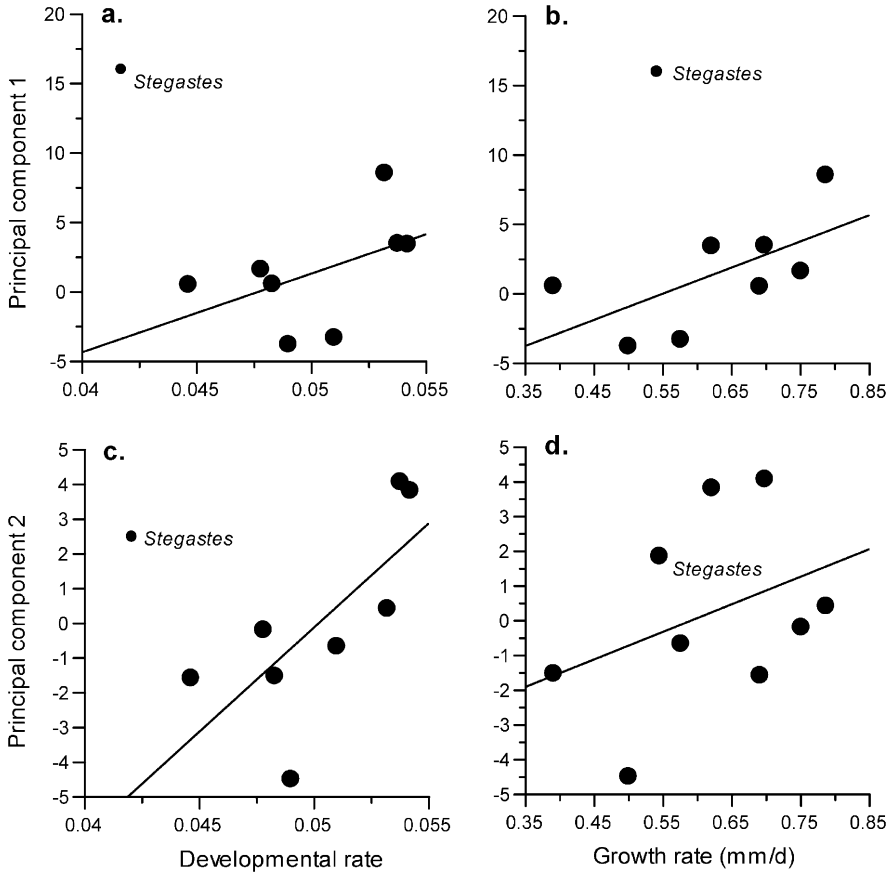

Fig. 6a–d The relationship between development rate, growth rate, and the first two principal components from an analysis of body change 3.5–4 days after capture in nine pomacentrid species. *Stegastes nigricans* has been labeled because it represents an outlier in graphs **a**, **b**, and **c** (see text) and has been excluded from the least-squares regression lines

mental rate ($r = 0.48$, Fig. 6a). A stronger relationship exists between PC 1 and growth rate averaged over the larval phase, such that the faster the growth rate the greater the change in snout angle at metamorphosis ($r = 0.63$, Fig. 6b). This relationship accounted for 40% of the variability in the data set.

A strong positive relationship was also evident between PC 2 (representing changes in pectoral fin and dorsal spine length) and developmental rate ($r = 0.71$, Fig. 6c). This suggests that fish species that develop fastest show the greatest change in pectoral fin and dorsal spine length. There was little relationship between PC 2 and larval growth rate (Fig. 6d).

Discussion

This study found that all 34 species of coral reef fish displayed a rapid change in either pigmentation, morphology, or both at the end of their larval phase. Morphological changes involved changes in the shape of the head, changes to the lengths of spines associated with dorsal and pectoral fins, and changes in body depth. An equal number showed regression and development of these features. Interestingly, it was not possible to generalize as to the types of changes that will occur at the end of the larval phase in fish at the family or genus level. This limited data set suggests that many of the changes may be species specific.

Most late larval stage fishes were cryptic in colouration, either having transparent muscle blocks, or silver and bronze sides with a dark ventral surface. The bright yellow pre-settlement bodies of the soapfish *Diploprion bifasciatum* and the boxfish, *Ostracion cubicus*, appear to defy the rule that larvae should be cryptic in colouration. It is interesting to note, however, that as adults both are known to secrete poisons from their skin when stressed. The bright yellow colouration may indicate the early development of this chemical defence mechanism.

All species showed a conspicuous change in pigmentation concomitant with settlement. Some species, such as the holocentrids, had conspicuous markings on their fins when first caught. Similarly, goatfishes (*Upeneus tragula*) in the first stages of caudal and dorsal fin pigmentation are sometimes captured amongst schools of earlier-stage larvae (M.I.M., personal observation). This may be a preparatory change due to upcoming settlement. Alternatively, fin colouration could be important for signaling while in the pelagic life stage. Leis and Carson-Ewart (1998) found complex behaviours during schooling in late larval stage fishes.

The extent to which settlement to a benthic environment and metamorphosis are linked in time is highly variable amongst coral reef fish taxa. The sergeant major *Abudefduf saxitilis* metamorphoses in the pelagic and settles to the reef with a full juvenile morphology and pigmentation at a broad size and age range (M.I.M.,

unpublished data). In contrast, the dartfish *Pteroleotris evides* undergoes a series of morphological changes over a 3-week period after settlement until a stable juvenile morphology is attained (McCormick and Makey 1997). The link between settlement and metamorphosis can only be assessed by having a full ontogenetic series of specimens from which the production of new structures and the regression of old can be determined. From assessments of the ontogeny of tropical larvae to date (see Leis and Carson-Ewart 2000) it can be noted that in general, when fish come into the vicinity of the reef to settle they are well on the way to losing specializations that they possessed for larval life. For instance, the second dorsal spine and pelvic fin spines that are conspicuous in serranids of the genera *Epinephelus* and *Plectropomus* are largest in *P. leopardus* at 7 mm total length (TL) at about 70–90% and 60–70% of body length, respectively (Masuma et al. 1993). This is well before settlement at about 20 mm TL. Likewise, McCormick (1999) found that the second dorsal spine showed a dramatic regression after settlement in *Acanthurus triostegus* (approximately 24 mm SL), but the relative size of this spine peaked in length in the mid-larval phase at about 12–17 mm SL (Leis and Carson-Ewart 2000). Compared to the development and regression of novel structures that has occurred to reach a stage of settlement competence, the changes in morphology that occur around settlement often represent the loss of relatively minor pelagic specializations.

Spine-length changes of greater than 5% of the body length over 4 days were a characteristic of 9 of the 34 species examined. Half of these exhibited reductions, with the greatest displayed in one specimen of the soapfish, *D. bifasciatum*, who lost banners from the second and third dorsal spines greater than 1.5 times its body length. The presence of spines on the deepest part of the body may make the larvae "effectively larger, painful to ingest, thus more resistant to predation" (Moser 1981). They may also play a role in buoyancy by greatly increasing the surface area, although this particular species has a well-developed swim bladder (Baldwin et al. 1991). Elaborate spinal banners such as these may also serve to distract predators or mimic siphonophores or salps (Govoni et al. 1986; Baldwin et al. 1991). Baldwin et al. (1991), who managed to rear *D. bifasciatum*, noted that the banner was both innervated and vascularized and they surmised that it may serve a sensory function.

Fish studied here commonly showed changes in the proportions of the head with marked changes (increases and decreases) in the angle of the snout. The commonality of this change is likely to be associated with diet changes that often occur with settlement. The temperate reef fish *Odax pullus* was found to display changes in head shape (i.e. jaw and gape size) with a change in diet from carnivorous to herbivorous at settlement and metamorphosis (Clements and Choat 1993). The Dover sole lose teeth that are replaced with others only on the right side of the body when they settle (Markle et al. 1992). Labelle and Nursall (1985) found that at settlement the Caribbean blenny, *Ophioblennius atlanticus*, change from being a pelagic predator with long fangs to being a grazing herbivore with fine comb-shaped teeth for nipping and scraping algal turf and bacteria. The change is estimated to take about a week to complete during which the fish lose about 7% of their length.

Some species have been shown not to feed during this period of remodeling. A non-trophic period over metamorphosis may be the reason underlying the body shrinkage found in six species of fishes, including two acanthurids, in the present study. Randall (1961) found no food in the guts of 30 newly recruited *Acanthurus triostegus*, suggesting that this species does not feed during this remodeling phase. Nursall and Turner (1985) suggest that the changes to the head and gut morphology that occur in the blenny, *O. atlanticus*, were fueled by reserves in the liver, which is large and fat laden at the start of metamorphosis and small as juveniles. Generally little is known of the energetics of metamorphosis in species other than those with leptocephalus larvae, such as the bonefish *Abula* (Pfeiler and Luna 1984; Pfeiler 1996; Pfeiler et al. 1998).

Contrasting morphological changes were found between species of the same genus and family. Differences in the changes that occur at settlement for *Pomacentrus amboinensis* and *P. bankanensis* suggest quite different preparations for settlement into the benthic environment. This study emphasizes the species-specific nature of the changes that occur around settlement and highlights the problem of generalizing morphological changes in demersal fish families.

Our results suggest that within the Pomacentridae, those species with a shorter larval duration, which develop faster through the larval phase, undergo a greater metamorphosis than those that spend more time in the plankton. This suggests that there may be a minimum morphological configuration for a fully functional benthic pomacentrid. Those species that spend more time as larvae have already developed a number of the pre-adaptations for their benthic existence whereas those with short PLDs are yet to develop fully. Interpretation of the analyses suggests that these changes involve changes in the shape of the head and reduction in spination. Why *Stegastes* is so different from the other pomacentrids examined in this regard is unclear. The difference may stem from differing phylogenies, which are presently unresolved.

Whereas the changes in pigmentation around settlement are often dramatic in tropical reef fishes, the morphological changes are usually subtle relative to the changes they have already undergone during larval development. These changes often include modification of feeding structures when settlement involves a trophic change. Although changes can be subtle, newly settled fish will be competing with conspecifics that have fully attained the specializations that allow them to use benthic resources efficiently. In evolutionary time this selective pressure may have resulted in newly settled fish

using slightly different habitat at settlement from juveniles. The limited evidence available to date suggests that there may be a relationship between the amount of post-settlement morphological change necessary to attain a juvenile form and the use of a number of transitional habitats immediately post settlement (McCormick and Makey 1997).

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