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Prevalence of ontogenetic changes in colour brightness among benthic invertebrates and their association with microhabitat shifts

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ABSTRACT: This study examined 15 species of intertidal invertebrates to determine the prevalence of ontogenetic changes in body colour brightness, the influence of diet and sunlight on body colouration, and the relationship between shifts in brightness and in microhabitat use. Most species did not undergo substantial changes in brightness or pigmentation. Some degree of change could be detected by image analysis software in 11 of the 15 species, but changes in brightness sufficient to be detected by a human observer occurred in only 6 species, and changes in pigmentation were apparent in only 3 species. In a controlled experiment, shell brightness and pigmentation of hatchings of the snail *Nucella ostrina* were found to be phenotypically plastic, changing in response to exposure to sunlight but not to diets of mussels or barnacles. Ontogenetic shifts in brightness were not phylogenetically constrained, occurring in the 3 phyla represented in our study (Mollusca, Annelida, Arthropoda). Shifts in brightness and pigmentation to increasingly darker shells or exoskeletons were found to occur in all species that shifted from cryptic to exposed microhabitats during ontogeny; the size at which colour brightness shift occurs is closely matched with the size at which microhabitat shift occurs in 3 species for which such information is available. However, little or no ontogenetic shift in brightness was detected in motile species with partial or no microhabitat shift. The ontogenetic timing of colour shifts may therefore serve as a marker of a transition between ecologically distinct phases of life.

KEY WORDS: Ontogeny · Predation · Camouflage · Distribution · Body colouration · Molluscs · Crustaceans · Life history

INTRODUCTION

Body colouration plays a key role in survival for many animals (Endler 1978, Burtt 1981, Booth 1990, Auerswald et al. 2008). In intertidal and shallow marine habitats, body colouration can serve a range of functions, such as protection from ultraviolet radiation (UVR), thermal resistance, sexual selection, and perception by visual predators (Reimchen 1979, Palmer 1985, Booth 1990, Palma & Steneck 2001, Bandaranayake 2006, Todd et al. 2006, Manriquez et al. 2009, Anderson et al. 2013). The nature of these interactions with the environment, however, can change substantially during juvenile and adult life, resulting in ecologically distinct stages of life (Werner & Gilliam 1984, Gosselin 1997). Accordingly, survival and growth through each ecological stage might be enhanced by the use of distinct, context-relevant body colourations during each stage (Booth 1990, Gosselin 1997, Palma & Steneck 2001, Todd et al. 2009, Anderson et al. 2013). For example, it has been suggested that visual crypsis (camouflage colouration) as well as behavioural crypsis (use of structural refuges) should be common in

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small-sized animals that are highly vulnerable to predators but much less so in larger-sized animals, as these are considerably less vulnerable to predators and thus are less needing of crypsis (Gosselin 1997, Palma & Steneck 2001, Anderson et al. 2013). If so, then body colouration and microhabitat use might be expected to change during ontogeny, and both changes might occur at the same age or body size. To date, however, too few species have been examined to test such hypotheses, and the limited evidence of ontogenetic shifts in body colouration that does exist comes from studies reporting on 1 or 2 species in which an ontogenetic shift in colouration was observed. In fact, it is not even known if ontogenetic changes in body colouration are common among benthic invertebrate species or whether such changes are perhaps noticeable but rare exceptions. In addition, even the mechanisms controlling the timing of ontogenetic shifts in body colouration are not well understood (Brake et al. 2004, Bandaranayake 2006). Studies to date have revealed that body colouration can be influenced by exposure to light and UVR (lobsters: Tlusty et al. 2009; shrimp: You et al. 2006) or by diet (limpets: Lindberg & Pearse 1990; snails: Manriquez et al. 2009). Since both factors can change when an individual changes microhabitat, these might constitute cues that determine the timing of ontogenetic shifts in body colouration.

This study sought to determine the extent to which ontogenetic changes in body colouration are common among intertidal invertebrates, and to clarify the mechanisms and adaptive significance of these changes. To do so, we focused on intertidal species with hard external body structures, specifically those producing a shell or exoskeleton. Such species represent a large proportion of the diversity of benthic invertebrate communities in many coastal regions of the world, including our study area. In addition, these species are amenable to the study of body colouration, being easier to handle without damage than soft-bodied animals (especially small juveniles), and the colours of shells and exoskeletons are relatively stable over at least the few hours or days required for field collection and colour analysis in the laboratory. Prior to this study, our casual observations of intertidal and shallow subtidal invertebrates over the last 2 decades had suggested that, although colour hue can vary extensively among individuals of a species, in most species the individuals retain roughly the same hue throughout their benthic life; when changes were observed, they most often involved individuals becoming lighter or darker with increasing size — a perception that was reinforced during the present study. We therefore quantified brightness (Booth 1990, Krause-Nehring et al. 2010), a feature also referred to as lightness or luminescence (Tlusty 2005, Tlusty et al. 2009), rather than hue. The analysis of brightness also has 3 additional advantages: enabling standardized comparisons among species, being relevant to all visual predators including those lacking full colour vision, and influencing absorptivity of solar radiation and thus being relevant to photoprotection and thermal resistance.

The specific goals of this study were to (1) determine the proportion of intertidal invertebrate species with hard external body coverings that undergo ontogenetic changes in brightness by examining 8 mollusc, 2 annelid and 5 arthropod species; (2) determine if ontogenetic shifts in brightness are associated with phylogeny (cf. phylogenetic inertia; Booth 1990), motility, or use of cryptic microhabitats; and (3) determine if the ontogenetic changes in shell pigmentation in the intertidal gastropod *Nucella ostrina,* reported by Gosselin (1997), can be caused by diet or exposure to sunlight.

MATERIALS AND METHODS

This study was carried out in Barkley Sound and at the Bamfield Marine Science Centre, on the west coast of Vancouver Island, Canada, from June to August 2012. All collections and transect surveys described below were carried out at the following 8 field sites: Scott's Bay (48° 50.2′ N, 125° 8.6′ W), Grappler Inlet (48° 49.9′ N, 125° 7.1′ W), entrance to Grappler Inlet (48° 50.3′ N, 125° 8.0′ W), Dixon Island (48° 51.2′ N, 125° 7.1′ W), Robbers Pass (48° 53.8′ N, 125° 7.3′ W), Fleming Island (48° 52.7′ N, 125° 9.7′ W), Ross Islets (48° 52.4′ N, 125° 9.6′ W), and Prasiola Point (48° 49.1′ N, 125° 10.1′ W).

Proportion of species that undergo ontogenetic changes in brightness

To determine the proportion of benthic invertebrate species that undergo ontogenetic changes in brightness, 15 intertidal species were examined (Table 1). Species were chosen for this study based on the following criteria: having a hard external body covering (calcareous shell or exoskeleton), being abundant in rocky intertidal habitats, being present in a broad range of sizes at the time of the study, and

Table 1. Species examined for ontogenetic changes in brightness. Motility: M = motile; S = sessile. Collection sites: SB = Scott's Bay; RP = Robbers Pass; FI = Fleming Island; RI = Ross Islets; GI = Grappler Inlet; PP = Prasiola Point; DI = Dixon Island; EGI = Entrance to Grappler Inlet

Phylum	Species	Type	Motility	Sample size	Collection sites
Mollusca	*Lirabuccinum dirum*	Snail	M	134	SB
	Littorina scutulata	Snail	M	112	SB
	Littorina sitkana	Snail	M	139	SB, RP, FI
	Nucella lamellosa	Snail	M	111	RI, GI
	Nucella canaliculata	Snail	M	87	PP
	Chlorostoma funebralis	Snail	M	126	SB
	Petaloconchus compactus	Vermetid	S	80	GI, DI
	Mytilus trossulus	Bivalve	M, S[a]	97	SB
Arthropoda	*Balanus glandula*	Barnacle	S	101	SB
	Chthamalus dalli	Barnacle	S	92	SB
	Pagurus hirsutiusculus	Hermit crab	M	95	SB, GI
	Pagurus granosimanus	Hermit crab	M	92	SB, GI
	Petrolisthes cinctipes	Crab	M	93	SB
Annelida	*Serpula columbiana*	Tubeworm	S	59	DI, EGI
	Spirorbis bifurcates	Tubeworm	S	83	DI

[a]In *M. trossulus*, juveniles are motile up to ~3 mm shell length, after which they become sessile

representing 3 distinct phylogenetic lineages (Mollusca, Annelida, Arthropoda) and 2 levels of motility during benthic life (sessile, motile). Animals were identified to species according to Kozloff (1996). Our samples of 2 species may have included specimens of morphologically similar species: samples of *Littorina scutulata* may have included specimens of *L. plena* (Mastro et al. 1982), and samples of small *Pagurus hirsutiusculus* may have included specimens of *P. holmi* (Ng & McLaughlin 2009).

Animals of a broad range of sizes were collected for each species. All animals were collected by hand, brought back live to the laboratory, and placed in a tank with flowing seawater. Within 48 h of collection, individuals were removed from the seawater tank, blotted dry, and weighed using a digital scale to the nearest 0.001 mg for animals <1000 mg, and to the nearest 0.01 mg for animals >1000 mg. Hermit crabs, however, were euthanized by freezing, carefully removed from their shells, rehydrated in salt water for 2 min, blotted dry, and weighed without their shell.

Brightness was quantified using an approach similar to that of Tlusty (2005) and Krause-Nehring et al. (2010). An image was taken of each animal under a dissecting microscope with a top-mounted digital camera (Olympus Model QCOLOR5). All specimens were illuminated using a halogen fibre optic light source, and care was taken to ensure the orientation of the animals and the lighting were the same for all images. All images were analyzed using Adobe Photoshop Elements 2.0. This approach was used to analyze the brightness of shells and exoskeletons and also to analyze the brightness of a standard backing material to verify that the imaging process was consistent, as described further below. For shells and exoskeletons, a single brightness value was obtained for each specimen; in shells, this value was obtained from the area of most recent growth, and thus least eroded, damaged or overgrown by biofilm or other organisms. To quantify shell brightness, we analyzed the area along the lip of the aperture of snails and tubeworms, near the posterior (siphonal) margin of the shell of mussels, and at the base of lateral plates of barnacles. For hermit crabs, we analyzed the upper surface of their right claw because these are generally the most exposed and visible parts of the body, an approach also used by Tlusty et al. (2009) for lobsters. For crabs, we analyzed the entire carapace except for the largest *Petrolisthes cinctipes* with carapaces that were too large to include in a single image under the microscope; in the latter cases, 4 to 7 images were taken in different, haphazardly selected regions of the carapace, analyzed separately, and then averaged. For all images, occasional spots of reflecting glare were de-selected and thus not included in the image analysis. All images were converted to grayscale, and then average brightness of the selected areas was quantified using the Mean Luminosity value in the Histogram function of Photoshop (Krause-Nehring et al. 2010). These mean luminosity values constitute an index of brightness ranging from 0 to 255, with 0 representing pure black and 255 representing pure white. The average brightness value of the 10 smallest individuals was compared with that of the 10 largest individuals for each species using a *t*-test, and species were assigned to one of 3 categories: (1) no significant change in brightness; (2) significant change in brightness <10%; and (3) significant change in brightness ≥10%. The 10% threshold (i.e. a dif-

ference of at least 25 points on the brightness index) were most likely to be biologically significant because changes ≥10% were readily detectable to the human eye; changes <10% could be detected by the digital imaging equipment, but were marginally or not detectable by the human eye. In addition to the above brightness measurements, we also examined by eye the shells and exoskeletons of each species to determine if changes in brightness were caused by changes in pigmentation.

To verify that lighting, exposure, and image analysis of shells and exoskeletons was consistent across the full range of sizes of a same species, we analyzed the brightness of the background material in images of the 10 smallest and 10 largest individuals of 4 species: *Mytilus trossulus, Littorina scutulata, Pagurus granosimanus,* and *P. hirsutiusculus*. When taking images of these species, each individual animal was placed on a grey plastic supporting plate (the same supporting plate being used for all individuals); parts of that plate were included in the image taken of each animal. We therefore compared average plate brightness in images of the 10 smallest individuals with that in images of the 10 largest individuals to test for consistency of the imaging process.

Published reports of the smallest size at settlement or hatching were found for 11 of the 15 species; according to those sizes, we were able to find and collect individuals that had just settled or hatched for 9 of these 11 species. Newly settled or newly hatched individuals of 2 species (*Chlorostoma* [formerly *Tegula*] *funebralis* and *Nucella canaliculata*) were not found at the time of this study. To our knowledge, the size at settlement or hatching is not known for 4 of the species in our study (*Littorina scutulata, Petaloconchus compactus, Serpula columbiana,* and *Spirorbis bifurcates*), so it is not clear if our samples included the smallest sizes for these species.

Relationship of brightness with phylogeny, motility and use of cryptic microhabitats

The occurrence of species with ontogenetic changes in brightness among phyla and among levels of motility was assessed based on the known phyletic lineage and motility of these species. Ontogenetic patterns of microhabitat use, however, are not known for most motile species; of the 10 motile species in our study, the ontogenetic pattern of microhabitat use has only been described for *Mytilus trossulus*. We therefore examined whether the use of cryptic microhabitats changes during ontogeny in 6 additional species: 4 snail species (*Lirabuccinum dirum, Chlorostoma funebralis, Littorina scutulata, L. sitkana*) and the 2 hermit crab species (*Pagurus granosimanus* and *P. hirsutiusculus*). Microhabitat use by these species was examined at Scott's Bay and Grappler Inlet. Time constraints prevented the examination of microhabitat use by *Nucella lamellosa, N. canaliculata,* and *Petrolisthes cinctipes*.

The approach used to assess patterns of microhabitat use was similar to that used by Gosselin & Chia (1995), although we modified the approach for hermit crabs. For snails, a 10 m transect line was positioned in the intertidal zone at low tide, parallel to the water line and within the range of intertidal heights occupied by each snail species. Microhabitat use was then examined within 25 × 25 cm quadrats placed at 2 m intervals along each transect line, with 5 quadrats per transect. All individuals found within the quadrats without disturbing the substratum or algae were collected; these individuals were considered 'exposed'. Then debris, rocks, and seaweed were slowly removed and any individuals hidden by these objects were collected, including those buried in sand or gravel; these were considered 'cryptic' individuals. Three transects and a total of 15 quadrats were sampled for each of *Lirabuccinum dirum* and *Littorina scutulata*. For each of *Chlorostoma funebralis* and *Littorina sitkana*, only 1 transect with 5 quadrats was analyzed due to time constraints. All collected animals were returned to the laboratory and weighed within the next 5 h, allowing us to determine the size frequency distribution of cryptic and exposed individuals.

During low tide, almost all hermit crabs of both species were found to be in cryptic microhabitats; crabs are much more motile than snails and can quickly travel longer distances and return to shelter within each tide cycle. At low tide, transect surveys were therefore carried out as described above for snails, but for the hermit crabs this served to document the overall size frequency distribution of the populations. The same habitat was then revisited at high tide, and all individuals found crawling on exposed surfaces were collected by standing in one location for 10 min and capturing all individuals that became visible using a dip net; these individuals were considered 'exposed'. Six different locations were sampled in this way. Given that these 2 hermit crab species coexist in the same intertidal areas (Straughan & Gosselin 2014), both species were sampled together during the low tide and high

tide surveys, and later identified to species in the laboratory.

Effect of diet and exposure to sunlight on shell brightness in *Nucella ostrina*

To determine if the onset of ontogenetic changes in brightness are triggered by external cues, we examined shell brightness in newly hatched *Nucella ostrina* in response to different light exposure and diet treatments. *N. ostrina* egg capsules were collected from Scott's Bay and Grappler Inlet when the young snails appeared close to hatching, i.e. when the capsule's plug was dissolved and hatchlings were clearly visible through the opening. These capsules were carefully detached from the rocks, brought back to the laboratory, and placed in containers with mesh siding in seawater trays. Hatchlings that emerged within 3 d of collecting the egg capsules were used in the following experiment.

Hatchlings were haphazardly assigned to one of 4 treatments: (1) exposed to sunlight and fed barnacles; (2) exposed to sunlight and fed mussels; (3) kept in the shade and fed barnacles; and (4) kept in the shade and fed mussels. In the barnacle-fed treatments, hatchlings were provided with small rocks colonized by *Balanus glandula* and *Chthamalus dalli*. In the mussel-fed treatments, hatchlings were fed small (1 to 3 mm SL) *Mytilus trossulus*. These prey species were offered because they are known to be important food sources for early benthic phase *Nucella ostrina* (Gosselin & Chia 1994, 1996). Cages containing the hatchlings were placed in a seawater tray that was partly covered by a sheet of opaque black cloth; half of the hatchlings were assigned to the shaded side of the tray (shade treatment), and half were assigned to sunlit side (sunlight treatment). Hatchlings were reared in these treatments for 21 d. Food was added every 2 to 3 d, as needed, and seawater temperature in the tray was checked daily. The design of this experiment was therefore as follows: 2 light treatments × 2 diet treatments × 7 replicate cages per treatment combination × 10 hatchlings nested within each cage. In addition, at the start of the experiment a separate set of 10 hatchlings from the same group of egg capsules were weighed and photographed to document the initial size and brightness of newly hatched individuals. At the end of the 21 d study period, live snails were individually weighed and photographed using the methods described earlier. Average shell brightness was compared among treatments using a 2-factor ANOVA.

RESULTS

Proportion of species that undergo ontogenetic changes in brightness

Brightness measurements of the grey plate used as background when photographing the animals revealed the imaging and image analysis process to be highly consistent across the range of magnifications and animal body sizes used in this study. The average brightness of plate material appearing in images of the 10 smallest individuals was not significantly different from that of plate material in images of the 10 largest individuals for 2 species: *Pagurus granosimanus* (2-sample *t*-test: $t = -1.352$, n = 20, df = 18, p = 0.194) and *Littorina scutulata* ($t = -0.848$, n = 20, df = 18, p = 0.408). Significant differences in plate brightness were detected in the 2 other species, *Mytilus trossulus* ($t = 2.426$, n = 20, df = 18, p = 0.026) and *P. hirsutiusculus* ($t = -4.874$, n = 20, df = 18, $p < 0.001$), but those differences were very small; average brightness differed by only 2.43 and 2.34 points on the 255 point scale, or 0.95 and 0.92%, respectively. These values demonstrate that the imaging and image analysis method used in this study provided accurate representations of shell and exoskeleton brightness and was in fact sufficiently precise and repeatable to detect differences as small as 0.9%.

A total of 1501 animals were individually examined for shell or exoskeleton brightness. Comparisons of average brightness values between the 10 smallest and 10 largest individuals of a given species revealed statistically significant differences in brightness in 11 of the 15 species (73%) (Table 2). Differences in average brightness between smallest and largest individuals of these species ranged from 2 to 29% (Table 2). It should be noted that percent differences shown in Table 2 were calculated as a proportion of the full 255 point brightness scale to allow interspecific comparisons. When differences in brightness are expressed as a proportion of the average brightness of the 10 smallest juveniles, an approach that is more familiar but less amenable to interspecific comparisons, the average brightness of the 10 largest adults differed from the smallest juveniles by as much as 50.4% (*Pagurus granosimanus*) and 70.7% (*Mytilus trossulus*). Differences in brightness between small and large sizes were not detected in 4 species (*P. hirsutiusculus, Lirabuccinum dirum, Littorina sitkana,* and *Nucella canaliculata*).

Among the 11 species in which brightness did differ significantly between the smallest juveniles and

Table 2. Comparisons of mean brightness between the 10 smallest and 10 largest individuals for each of the 15 species. MB = mean brightness; S = sessile; M = motile (during early juvenile life); * = significant; **bold** names indicate species in which mean brightness of the smallest and largest individuals differed by ≥10%

Phylum Subphylum/Class *Species*	Motility	MB of 10 smallest (±SD)	MB of 10 largest (±SD)	p (*t*-test)	Difference	Change in pigmentation
Arthropoda						
Maxillopoda						
Balanus glandula	S	106 ± 8	155 ± 10	0.001*	19% lighter	No
Chthamalus dalli	S	48 ± 3	59 ± 4	0.022*	4% lighter	No
Malacostraca						
Pagurus hirsutiusculus	M	38 ± 4	63 ± 6	0.171		No
Pagurus granosimanus	M	73 ± 4	37 ± 2	<0.001*	14% darker	Yes
Petrolisthes cinctipes	M	42 ± 2	28 ± 1	<0.001*	5% darker	No
Mollusca						
Gastropoda						
Lirabuccinum dirum	M	45 ± 5	45 ± 3	0.153		No
Littorina scutulata	M	26 ± 3	40 ± 3	0.005*	5% lighter	No
Littorina sitkana	M	36 ± 5	42 ± 6	0.394		No
Nucella lamellosa	M	153 ± 6	81 ± 14	0.03*	29% darker	Yes
Nucella canaliculata	M	86 ± 10	89 ± 8	0.832		No
Chlorostoma funebralis	M	34 ± 2	29 ± 1	0.007*	2% darker	No
Petaloconchus compactus	S	56 ± 6	118 ± 13	<0.001*	24% lighter	No
Bivalvia						
Mytilus trossulus	M, S[a]	62 ± 6	22 ± 4	<0.001*	16% darker	No
Annelida						
Polychaeta						
Serpula columbiana	S	159 ± 11	182 ± 8	0.005*	9% lighter	No
Spirorbis bifurcates	S	148 ± 11	196 ± 10	0.004*	19% lighter	No

[a]In *M. trossulus*, juveniles are motile up to ~3 mm shell length, after which they become sessile

largest adults, species differed in whether the adults were lighter or darker than the smallest juveniles. In 5 species, adults were darker than the smallest juveniles (*Pagurus granosimanus*, *Petrolisthes cinctipes*, *Nucella lamellosa*, *Chlorostoma funebralis*, and *Mytilus trossulus*), whereas adults were lighter than the smallest juveniles in 6 species (*Balanus glandula*, *Chthamalus dalli*, *Littorina scutulata*, *Petaloconchus compactus*, *Serpula columbiana*, and *Spirorbis bifurcates*). Of the 11 species in which average brightness differed significantly between large and small body sizes, modest differences (<10%) were observed in 5 species (Fig. 1), and more substantial differences (≥10%) were observed in 6 species (Fig. 2).

In 2 species, *Pagurus granosimanus* and *Mytilus trossulus*, brightness values decreased rapidly with increasing body size among the smallest juveniles, followed by stable brightness values among larger body sizes (Fig. 2B,E). In addition, the trend in both species was of increasingly darker shell or exoskeleton with increasing body size. A similar pattern was observed in *Nucella lamellosa*, with all newly hatched individuals having light coloured shells and most larger individuals being darker, although some *N. lamellosa* of all sizes have light-coloured shells (Fig. 2C). In all 3 species, differences in brightness corresponded to visible differences in density of pigmentation of the shell or exoskeleton. Trends of brightness as a function of body size were more gradual in other species and no differences in the colour or density of pigmentation were apparent.

Relationship of brightness with phylogeny, motility and use of cryptic microhabitats

Significant differences in brightness between small and large-bodied individuals were not restricted to a single phylogenetic lineage. Each of the 3 phyla included species exhibiting substantial differences (i.e. ≥10%) in brightness as well as species with modest or no differences (Fig. 3A). Significant differences in brightness were also recorded in motile as well as in sessile species (Fig. 3B). However, the trend in all 5 sessile species was for larger individuals to be lighter than smaller individuals, whereas in 5 of the

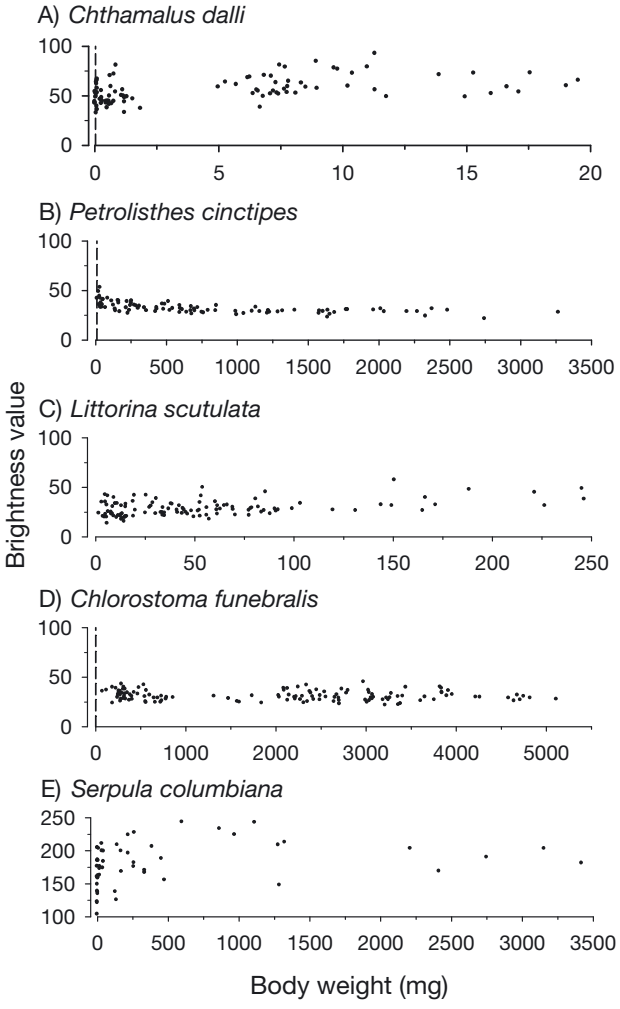

Fig. 1. Brightness of external body covering as a function of body weight (mg); species in which the brightness index measurements changed significantly with body size but by less than 10%. Vertical dashed line indicates literature values of the recorded hatching or settling size for that species

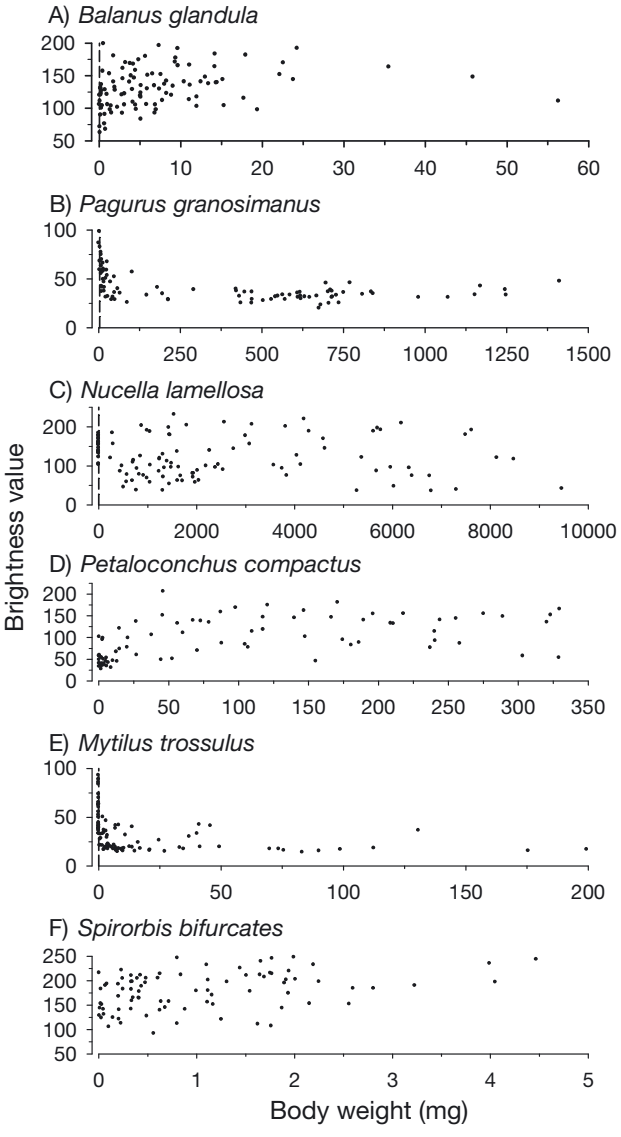

Fig. 2. Brightness of external body covering as a function of body weight (mg); species in which the brightness index measurements changed significantly with body size but by more than 10%. Vertical dashed line indicates literature values of the recorded hatching or settling size for that species

7 motile species, larger individuals were darker than smaller individuals (Table 2).

Pronounced differences in microhabitat use as a function of body size were apparent in only 1 of 6 motile species, the hermit crab *Pagurus granosimanus*. The proportion of *P. granosimanus* individuals remaining in cryptic microhabitats at high tide was 100% for the smallest individuals but dropped to less than 20% for the largest individuals (Fig. 4A). Modest differences in microhabitat use were apparent in 2 other species, *Lirabuccinum dirum* and *Littorina scutulata* (Fig. 4C,D); a majority of individuals in all size classes were found in cryptic microhabitats, but the proportions did decrease slightly from 100% of the smallest juveniles to 50–70% of larger individuals. In the 3 other motile species that were examined, the frequency of use of cryptic microhabitats was comparable among size classes (Fig. 4B,E,F). Among motile species (including *Mytilus trossulus* and *Nucella ostrina* for which microhabitat shifts have been described elsewhere), those in which microhabitat use differed among size classes were the only ones found to also exhibit differences in in brightness (Fig. 3C) and in pigmentation as a function of body size. It should be noted, however, that a modest number of

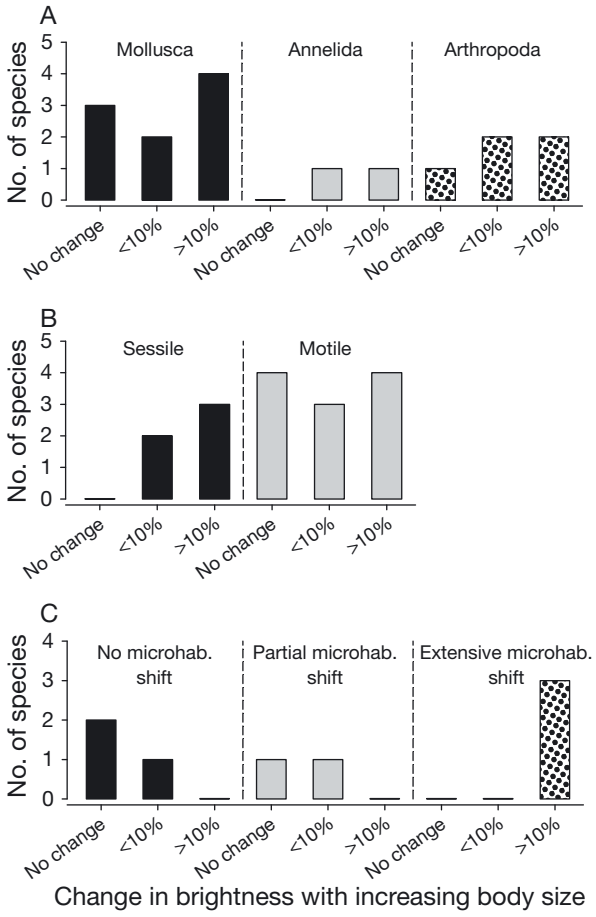

Fig. 3. Number of species in each category of brightness change, grouped according to (A) phylogeny, (B) motility, and (C) whether microhabitat use changes during ontogeny (motile species only). (A), (B) and (C) include the gastropod *Nucella ostrina,* a motile mollusc with substantial ontogenetic changes in brightness and in microhabitat use (Gosselin 1997), and (C) also includes *Mytilus trossulus,* a species with an extensive microhabitat shift (Jenewein & Gosselin 2013)

Littorina sitkana were sampled in this survey, and no large individuals were present at that time.

Effect of diet and exposure to sunlight on shell brightness in *Nucella ostrina*

Experimental rearing of *Nucella ostrina* hatchlings for 21 d revealed significant differences in shell brightness between light treatments but not between diet treatments (Table 3). Shells of the 21 d old juvenile snails were 28% darker in the sunlight treatment than in the shade treatment (Fig. 5). The light treatments did not differ, however, in terms of snail survivorship (Student's *t*-test, Arcsin transformed data (Zar 2010): $t = 0.84$, df = 25, n = 28, p = 0.409) or final body size (*t*-test: $t = 1.94$, n = 27, df = 23, p = 0.069); on Day 21, average survivorship was 45.7 ± 14.0% (average ± SD), and surviving snails had grown in body weight by 61.0 ± 44.8%. At the end of the experiment, one cage (shaded, barnacles) contained only 1 live snail and one other cage (exposed, barnacles) contained only 2 live snails; all other 26 cages contained 3 to 7 live snails.

DISCUSSION

For those species in which brightness changed substantially (≥10%) with increasing body size, 2 lines of evidence suggest these changes were the result of ontogenetic shifts rather than differential mortality. Firstly, in species that produce a shell, the shell itself is a record of current and past colouration of the individual; in these species, patterns among body sizes of brightness and pigmentation that were revealed by our survey were also clearly visible within the shells of individuals. Secondly, in most species with substantial changes, the brightness values measured in mid-sized and large individuals were not present in the smallest individuals of the same species (e.g. *Pagurus granosimanus*; Fig. 2B). We are therefore confident that the patterns of brightness reported in this study represent ontogenetic trends in these species.

Proportion of species that undergo ontogenetic changes in brightness

Significant ontogenetic changes in brightness occurred in several intertidal invertebrates that produce a shell or exoskeleton, but the degree of change varied broadly among species. Changes were detected in 11 of the 15 species (73%) examined in this study, with average differences between the smallest and largest individuals ranging from as little as 2% to up to 29% of the 255 point brightness scale. Substantial (≥10%), and potentially biologically significant, changes in brightness were apparent in only 6 species. In addition, ontogenetic changes in pigmentation were only visible in 3 of the 15 species: *Pagurus granosimanus*, *Mytilus trossulus* and *Nucella lamellosa*. Our observations of the 8 other species with significant changes in brightness suggests changes in these species were largely or entirely due to a thickening of the shell or exoskeleton early in juvenile life. Shortly after metamorphosis, the early shell or exoskeleton is often very thin and translucent; less light is reflected from the surface, causing their brightness

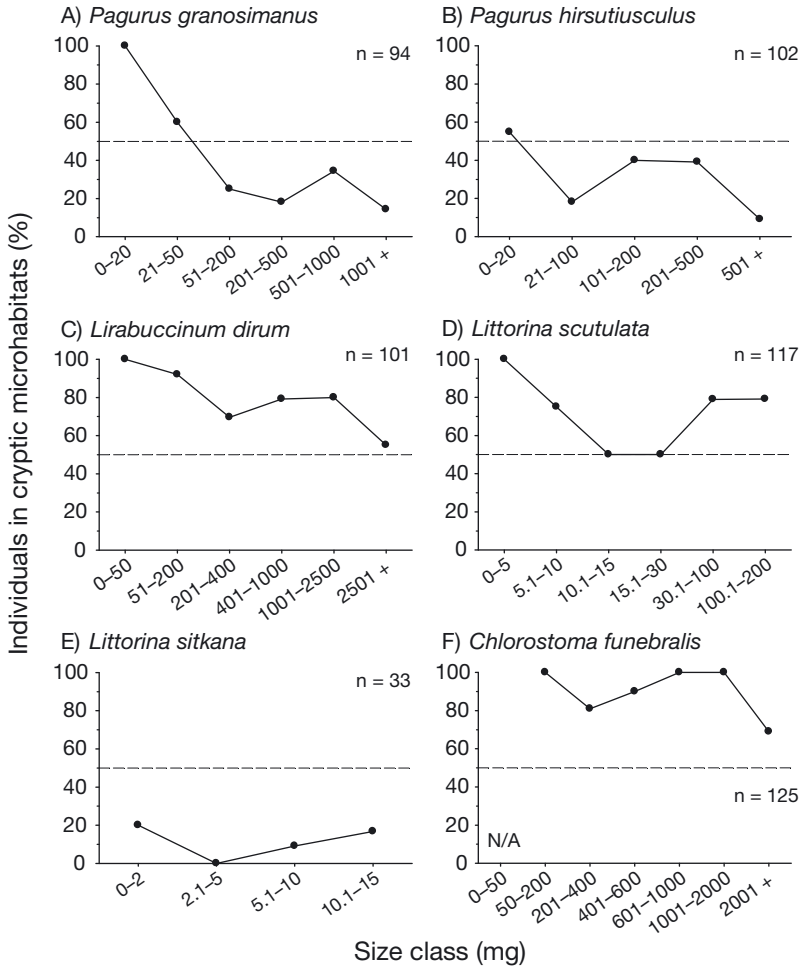

Fig. 4. Microhabitat use survey. Proportion of individuals using cryptic microhabitats in the field as a function of body size in 6 motile species. Dashed horizontal line is a reference line indicating 50% of individuals

Table 3. Nested 2-way ANOVA (Zar 2010) of body brightness index for *Nucella ostrina* hatchlings in light and diet treatments; n = 28 replicate cages

Source	df	MS	*F*	p
Light	1	33150	60.42	<0.001
Diet	1	236	0.43	0.518
Light × Diet	1	148	0.27	0.608
Residual	24	549		

value to be low (i.e. dark), but as the animal grows it gradually secretes a thicker shell or exoskeleton which becomes more opaque and reflects more light. This effect was most pronounced in 3 species that produce white shells (*Balanus glandula*, *Spirorbis bifurcates* and *Serpula columbiana*) and 1 species producing light purple shells (*Petaloconchus compactus*).

Relationship of brightness with phylogeny, motility and use of cryptic microhabitats

Each of the 3 phyla represented in this study (Mollusca, Annelida, and Arthropoda) included at least 1 species with extensive changes in brightness and at least 1 species with moderate or no change. In addition, ontogenetic changes in pigmentation of the shell or exoskeleton were apparent in species from 2 phyla (Mollusca and Arthropoda). Changes in pigmentation were not observed in annelid species, but this may simply be a consequence of our small sample size of 2 species for this phylum. Overall, these findings indicate that ontogenetic shifts in brightness and pigmentation are not phylogenetically constrained.

During life in the benthos, from newly established individual to full-sized adult, motile individuals have the opportunity to change microhabitat, as well as their diet and their exposure to environmental factors such as sunlight, desiccation, wave action and predators. Sessile individuals, however, experience roughly the same set of conditions from the moment of attachment onwards. Accordingly, motile animals would seem more likely than sessile species to benefit from displaying a different appearance at different periods of benthic life. However, brightness did not change in 4 of the motile species, and some change in brightness did occur in all 5 sessile species. There were, nevertheless, some differences between sessile and motile species. A trend of darker shells or exoskeletons with increasing body size was only observed in motile species; all 5 sessile species became lighter in colour. In addition, ontogenetic changes in the pigmentation of shell or exoskeleton were only apparent in motile species. Our results therefore provide only partial support for a link between motility and ontogenetic changes in brightness. This is probably because while motile species have the opportunity to experience different environmental conditions during ontogeny, not all of them do. Of the 6 motile species in which the use of cryptic and exposed microhabitats was examined, a pronounced ontogenetic shift was only observed in *Pagurus granosi-*

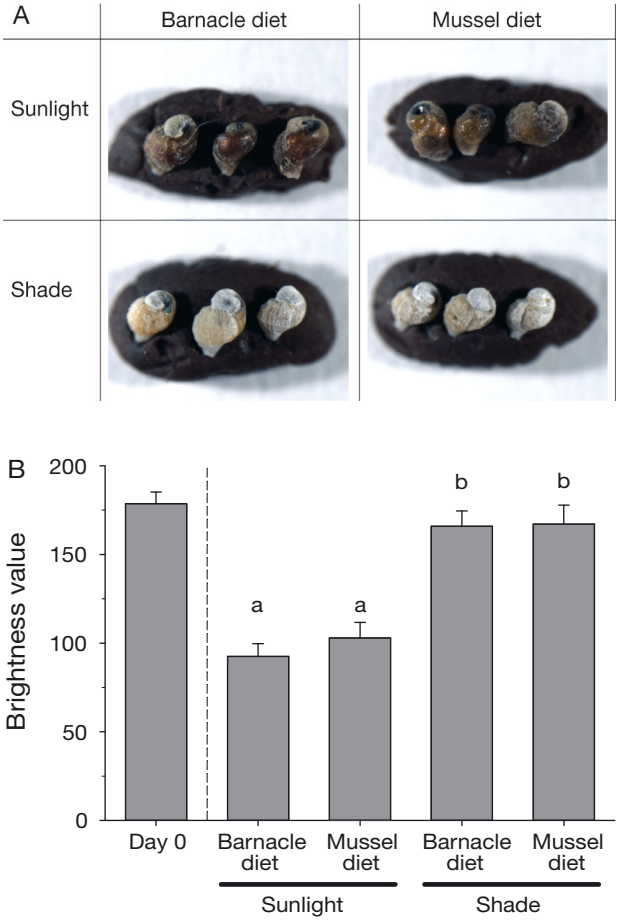

Fig. 5. Light and diet experiment. (A) Photographs revealing shell brightness of juvenile *Nucella ostrina* from the light exposure and diet treatments, taken at the end of the experiment on Day 21. (B) Brightness index of new shell growth of *Nucella ostrina* juveniles in the light and diet experiment. Day 0 = start of experiment (n = 10); all other values measured at end of the 21 d experiment. Letters above each bar identify groups of values that are not significantly different based on Tukey LSD test. Error bars show SE

manus; partial or no ontogenetic shift in microhabitat use was observed in the 5 other species, although a definitive verdict cannot be reached for *Chlorostoma funebralis* because individuals <50 mg were not present at the time of our study.

Ontogenetic patterns of microhabitat use have only been examined in a small number of benthic invertebrate species to date. Nevertheless, we do find a consistent association between microhabitat shift and colour shifts in those species that have been examined. Substantial ontogenetic shifts in brightness, pigmentation, and in use of cryptic microhabitats occurred in the hermit crab *Pagurus granosimanus*. Ontogenetic shifts from cryptic to exposed microhabitats have also been reported in *Mytilus trossulus* (Jenewein & Gosselin 2013) and in *Nucella ostrina* (Gosselin 1997), and changes in brightness and pigmentation with increasing body size occur in both species (*M. trossulus*, this study; *N. ostrina*, Gosselin 1997). We also found substantial changes in brightness and pigmentation in *N. lamellosa*; the ontogeny of microhabitat use by this species has not yet been formally examined, but during years of fieldwork in the same study area (L. A. Gosselin pers. obs.), medium to large-sized *N. lamellosa* were commonly observed on open surfaces whereas small juveniles were not, suggesting a shift from cryptic to exposed microhabitats in species as well. In addition, ontogenetic changes in carapace brightness and in use of cryptic microhabitats occur in the crabs *Cancer productus* (in our study area; Krause-Nehring et al. 2010), as well as *C. irroratus* (Atlantic coast of North America; Palma & Steneck 2001) and *Carcinus maenas* (UK; Hogarth 1978, Todd et al. 2006). In all 7 species listed above, distribution shifted from cryptic to exposed microhabitats and shells or exoskeletons were increasingly dark with increasing body size. Species with partial or no shift in microhabitat use (*Pagurus hirsutiusculus, Lirabuccinum dirum, Littorina scutulata, Littorina sitkana*) also had little or no ontogenetic shift in brightness.

Effect of diet and exposure to sunlight on shell brightness in *Nucella ostrina*

In the field, newly hatched *Nucella ostrina* mostly consume bivalve prey, and strongly prefer the mussel *Mytilus trossulus* over all other prey species (Gosselin & Chia 1994, 1996). Barnacles such as *Balanus glandula* and *Chthamalus dalli* are added to their diet ~2 to 4 mo after hatching, when the young snails leave structurally complex microhabitats and begin to explore open rock surfaces where barnacles are found (Gosselin & Chia 1995). However, this dietary transition would not cause changes in shell brightness in juvenile *N. ostrina*, as brightness at the end of our experiment was the same regardless of whether juveniles had fed on mussels or barnacles. This response to diet contrasts sharply with that of another predatory intertidal snail from the same family (Muricidae), *Concholepas concholepas*, in which early juveniles grow light or dark coloured shell as a result of feeding on barnacles or mussels, respectively (Manriquez et al. 2009). Shell brightness did change in *N. ostrina*, however, in response to light exposure, with snails in our experiment adding dark pigments

to new shell growth and becoming 28% darker when exposed to bright sunlight. This change was induced when the snails were still <0.68 mg in body weight, well below the size (~3 mg) at which shell colouration would normally begin to change in the field (Gosselin 1997).

Although few studies to date have examined the role of light as an inducer of changes in pigmentation in benthic invertebrates, colour change has been associated with exposure to light in some species. In the American lobster *Homarus americanus,* the pigmentation of its exoskeleton becomes darker when exposed to broad-spectrum or ultraviolet light (Tlusty et al. 2009), and tissue pigmentation also increases in the shrimp *Litopenaeus vannamei* in response to exposure to bright light (You et al. 2006). These studies demonstrate the responsiveness of body colouration to diet or light exposure in marine invertebrates and suggest these 2 factors might also play a role in the timing of ontogenetic shifts in body colouration.

Ecological and evolutionary implications

Of the 11 species with statistically significant changes in brightness, the changes observed in 8 species occurred primarily as a result of structural changes of the external body covering without noticeable changes in pigmentation. The brightness changes in these species appear to be an indirect consequence of the process of producing a very thin, translucent initial shell or exoskeleton at the start of the early benthic phase and then progressively growing a thicker and more reflective structure. The implications of these changes, if any, for the survival, growth and health of the individual are not clear. Further work is needed to determine if the changes in brilliance observed in these species are adaptive.

In each of the 3 other species with significant ontogenetic changes in brightness, the changes corresponded to increasing pigmentation of the shell or exoskeleton and progressively darker colouration. All 3 species are also motile and at least 2 species (probably all 3) relocate from cryptic to exposed microhabitats during ontogeny. As reviewed above, similar changes in body colouration and in microhabitat use have been reported in at least 4 other species. When a benthic invertebrate relocates from a cryptic to an exposed microhabitat, its exposure to a range of environmental factors changes, including predators. A negative relationship exists between body size and predation risk in benthic invertebrates (Hannaford Ellis 1984, Gosselin 1997, Griffiths & Gosselin 2008, Anderson et al. 2013), and this has been proposed as a reason why small individuals of motile species are often located in cryptic microhabitats whereas larger individuals often are not. In a similar way, ontogenetic changes in body coloration have been proposed as a mechanism that reduces predation risk in small, vulnerable individuals by minimizing the contrast between their body and the surrounding habitat (Todd et al. 2006). As individuals grow, their ability to blend into their surroundings changes, which in turn may favour different body colours or patterns (Hacker & Madin 1991, Gosselin 1997, Todd et al. 2006 2009). In addition, the timing of colour brightness shifts appears to be synchronized with the timing of microhabitat shifts in the 3 species for which both shifts have been document to date. In *Pagurus granosimanus*, both shifts occurred over approximately the 1 to 50 mg range of body sizes (this study). *Mytilus trossulus* change microhabitats when they reach sizes of 2 to 3 mm shell length (~0.8 to 2.7 mg) (Jenewein & Gosselin 2013), and changes in shell brightness are complete by the time they reach a size of ~3 mg (this study). *Nucella ostrina* shift microhabitats over the 3 to 55 mg size range, and shifts in shell pigmentation occur over the same size range (Gosselin 1997). These findings support the hypothesis that body colouration and microhabitat use at the beginning of benthic life are a response to predation pressure and that the timing of ontogenetic shifts in these traits is linked to changes in vulnerability to predation. If correct, then the occurrence of substantial shifts to darker colouration could be used as a simple indicator of species that also undergo ontogenetic shifts in microhabitat use; the ontogenetic timing of these colour shifts may constitute an indicator marking the transition period between 2 ecologically distinct phases of life — the early benthic and late benthic phases.

Why shift to darker colouration? The significance of the shift to darker colouration is not entirely clear but does suggest thermal resistance is not a major function of adult colouration. A darker colouration might in fact exacerbate heat stress during low tide or, as demonstrated by Miller & Denny (2011) studying white and black littorine snails, brightness may have only a modest, if any, influence on thermal regime. Shifts to darker body colouration might serve in sexual selection in some species (Endler 1978, Detto 2007), but this would not explain shifts to darker colouration in species with limited vision, such as snails and bivalves. Alternatively, darker body coverings might serve to increase protection from UVR (Tlusty et al. 2009), to which individuals

become increasingly exposed as they shift from cryptic to open microhabitats. Further research is needed to clarify the function of body colour at each ontogenetic stage, especially with regards to the role of colour on vulnerability to UVR and predation.

Finally, adult body colour is under genetic control in at least some marine invertebrates (Palmer 1984, Ekendahl & Johannesson 1997, Brake et al. 2004, Tlusty & Hyland 2005, Bandaranayake 2006). The information available to date, however, suggests that in at least some species the timing of ontogenetic changes in pigmentation is not genetically fixed. It is likely that initial juvenile pigmentation as well as adult pigmentation are genetically determined, but that the timing of ontogenetic change in an individual is phenotypically plastic. The timing of the shift in pigmentation would be controlled by environmental cues that change when the individual begins the transition from cryptic to exposed microhabitats, such as exposure to sunlight (as shown here in *Nucella ostrina*) or diet.

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