

HETEROCHRONIC DEVELOPMENTAL SHIFT CAUSED BY THYROID HORMONE IN LARVAL SAND DOLLARS AND ITS IMPLICATIONS FOR PHENOTYPIC PLASTICITY AND THE EVOLUTION OF NONFEEDING DEVELOPMENT

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Abstract.—Recent work on a diverse array of echinoderm species has demonstrated, as is true in amphibians, that thyroid hormone (TH) accelerates development to metamorphosis. Interestingly, the feeding larvae of several species of sea urchins seem to obtain TH through their diet of planktonic algae (exogenous source), whereas nonfeeding larvae of the sand dollar *Peronella japonica* produce TH themselves (endogenous source). Here we examine the effects of TH (thyroxine) and a TH synthesis inhibitor (thiourea) on the development of *Dendraster excentricus*, a sand dollar with a feeding larva. We report reduced larval skeleton lengths and more rapid development of the juvenile rudiment in the exogenous TH treatments when compared to controls. Also, larvae treated with exogenous TH reached metamorphic competence faster at a significantly reduced juvenile size, representing the greatest reduction in juvenile size ever reported for an echinoid species with feeding larvae. These effects of TH on *D. excentricus* larval development are strikingly similar to the phenotypically plastic response of *D. excentricus* larvae reared under high food conditions. We hypothesize that exogenous (algae-derived) TH is the plasticity cue in echinoid larvae, and that the larvae use ingested TH levels as an indicator for larval nutrition, ultimately signaling the attainment of metamorphic competence. Furthermore, our experiments with the TH synthesis inhibitor thiourea indicate that *D. excentricus* larvae can produce some TH endogenously. Endogenous TH production might, therefore, be a shared feature among sand dollars, facilitating the evolution of nonfeeding larval development in that group. Mounting evidence on the effects of thyroid hormones in echinoderm development suggests life-history models need to incorporate metamorphic hormone effects and the evolution of metamorphic hormone production.

Key words.—Echinodermata, heterochrony, life-history evolution, metamorphosis, phenotypic plasticity, planktotrophy, thyroxine.

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A range of developmental strategies characterizes life-history patterns in echinoderms (sea urchins, sand dollars, starfish, and their kin) and other marine invertebrates (reviewed in Wray 1995; terminology sensu McEdward and Janies 1997). Planktotrophic development, as exemplified by the pluteus larva of sand dollars and the veliger larva of snails, represents one extreme in this spectrum in which larvae develop from relatively small, energy-poor eggs. The elaborate and highly efficient feeding structures in these larvae compensate for the fact that the eggs of planktotrophs do not contain sufficient maternally derived resources to complete development in the absence of food. Lecithotrophic development proceeds from much larger eggs. Since sufficient maternally derived reserves are available for lecithotrophic larvae, feeding structures are reduced or even absent. Most planktotrophic and lecithotrophic larvae then undergo a radical metamorphosis into a settled juvenile. Direct development is a special case of lecithotrophy in which the larval stage is completely lost and the embryo develops directly into the juvenile with no intervening metamorphosis. The presumed ancestral mode of development in echinoderms is planktotrophic, and it is generally accepted that lecithotrophy has evolved multiple times independently from planktotrophic ancestors through the loss of larval feeding (e.g. Strathmann 1978, 1985; Wray 1996; but see Lacalli 1993). Traditional life-history models and their modifications (Vance 1973a,b; Christiansen and Fenchel 1979; Roughgarden 1989; Havenhand 1995; McEdward 1997) have assumed that egg

size is the prime determinant of developmental mode, and that ecological factors explaining selection for increased egg size can account for the evolution of lecithotrophy. Mechanistic (ontogenetic) factors have received little attention in these models.

Planktotrophic echinoderm larvae, most famously those of echinoids (sea urchins and sand dollars, the main focus of this study), respond in a phenotypically plastic way to the abundance of food under laboratory and field conditions (e.g. Fenaux et al. 1985; Boidron-Metairon 1988; Strathmann et al. 1992; Fenaux et al. 1994; Hart and Strathmann 1994; George 1999). Planktotrophic echinoids develop via a feeding pluteus larva, which captures algae using the convoluted ciliary band that runs along its several pairs of long, skeletal arms (Fig. 1A). The juvenile rudiment (Fig. 1B) develops as an essentially separate entity from the larval body. When the larvae of several echinoid species are reared under high food conditions, a differential increase in the growth of juvenile structures relative to the growth of larval structures occurs. By contrast, under low food conditions, juvenile growth is delayed, and the larvae instead invest in longer larval arms (Boidron-Metairon 1988; Hart and Scheibling 1988; Strathmann et al. 1992; Fenaux et al. 1994; Hart and Strathmann 1994). This differential allocation of energy to larval versus juvenile structures under low food conditions has been shown to result in an increased ability to capture food items (Hart and Strathmann 1994), and is thus considered an example of adaptive phenotypic plasticity.

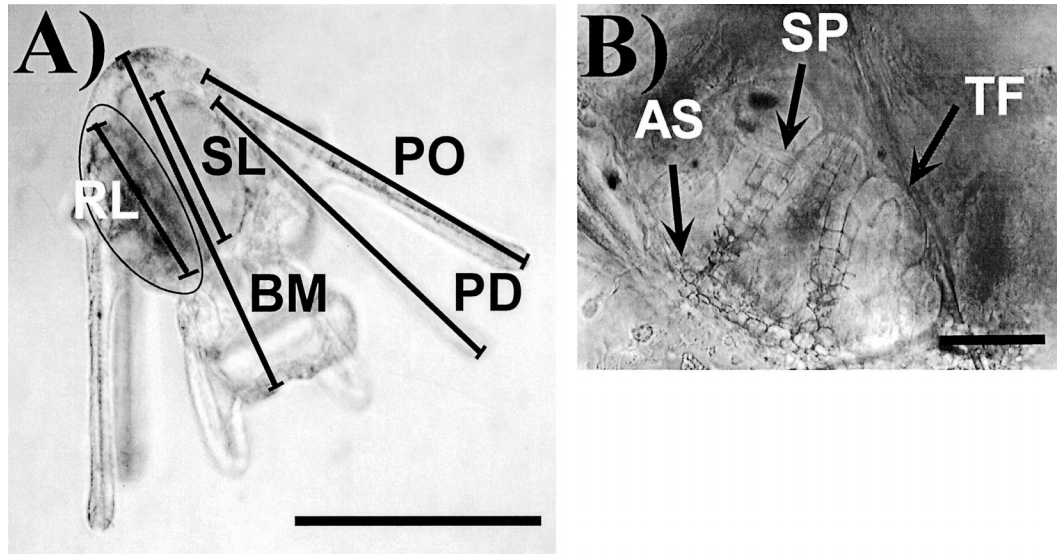


FIG. 1. Larval and adult characters analyzed in this study. (A) Pluteus larva and (B) close-up image of the juvenile rudiment (echinus rudiment; circled in A) in the planktotrophic larva of *Dendraster excentricus*. In the morphometrics and phenotypic plasticity experiments (see Materials and Methods), five larvae were randomly chosen and the indicated structures were measured for each replicate. PO, postoral arm; PD, postdorsal arm; BM, body midline; SL, stomach length (stomach size [SS] is a combined measure of length and width—width measured but not indicated; for details see Material and Methods); RL, juvenile rudiment length (juvenile rudiment size [RS] is a combined measure of length and width—width measured but not indicated; for details see Material and Methods); AS, adult skeleton; SP, juvenile or adult spine; TF, tube foot. PO, PD, BM, SL, and RL were measured; AS, SP, TF, and other adult structures not indicated here were used to stage the development of the juvenile rudiment (see Table 1). Bar in (A) equals 300 μm; in (B), 40 μm.

Strathmann et al. (1992) noted that planktotrophic echinoid larvae that develop from relatively large eggs exhibit a developmental trajectory superficially similar to that of larvae with smaller eggs that are raised under high food conditions: both tend to exhibit increased relative allocation to juvenile rather than to larval structures. These authors hypothesized that endogenous (maternally derived) and exogenous (derived from the plankton) food resources are essentially equivalent, and that the evolution of lecithotrophy, and the accompanying loss of larval feeding structures, could be explained simply by an evolutionary increase in maternal investment in the egg. However, later experiments with egg size manipulations (Bertram and Strathmann 1998; R. R. Strathmann, pers. comm. 2001) showed that alterations of exogenous and endogenous resources do not produce equivalent effects on larval morphology, contrary to the prediction of the aforementioned hypothesis.

An alternative hypothesis is that exogenous food is fundamentally different from endogenous food, or, more specifically, that planktotrophic larvae need to obtain specific compounds from planktonic algae that are not provided in the egg. A corollary to this hypothesis is that either lecithotrophic larvae synthesize these compounds themselves, or these compounds are supplied to the larvae maternally. Recent work on the mechanisms of echinoderm metamorphosis (Chino et al. 1994; Johnson and Cartwright 1996; Suyemitsu et al. 1997; Saito et al. 1998, Johnson 1998; Hodin et al. 2001) suggests that thyroid hormone may be one such compound.

Thyroid hormones (T4 [thyroxine] and T3 [3,3',5-triiodothyronine] and others) are best known for orchestrating



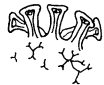

metamorphosis in amphibians: several thyroid hormone synthesis inhibitors (such as KClO₄ and thiourea) prevent metamorphosis, whereas exogenous hormonal applications cause precocious metamorphosis (reviewed in Rose 1999). Here we investigate the role of thyroid hormones in larval development and metamorphosis of the sand dollar *Dendraster excentricus*. We demonstrate that *D. excentricus* larvae do, indeed, respond to exogenous thyroid hormone (thyroxine) and a thyroid hormone synthesis inhibitor (thiourea) in a similar manner to the adaptive, plastic response of *D. excentricus* larvae to increased and decreased larval nutrition, respectively. We also present data suggesting that *D. excentricus* larvae can produce some endogenous thyroid hormone (or a thyroid hormone-like compound; but see Materials and Methods), but not enough to support development to metamorphic competence in the absence of food. We discuss our results in the context of phenotypic plasticity and life-history evolution in echinoderm larvae.

MATERIALS AND METHODS

We report on four experiments with larvae of *Dendraster excentricus* (Eschscholtz, 1831) from spring and summer 2000, 2001, and 2002: a morphometrics experiment, a phenotypic plasticity experiment, a metamorphosis experiment, and an inhibitor experiment. Sand dollars (*D. excentricus*) were collected at Crescent Beach, Orcas Island, Washington, at low tide in the spring and summer, held in flow through sea tables with preconditioned sand, and spawned later the same year.

We did not isolate or measure thyroid hormones in this study. Chino et al. (1994) measured thyroid hormone (T3

TABLE 1. Temporal occurrence of characters in the juvenile rudiment, and definition of rudiment stages. + indicates presence of juvenile character; - indicates absence of juvenile character. Note that body skeletal plates include first multibranching spicules (as indicated in drawing). See also Figure 1B for an image showing some of these juvenile characters.

Rudiment stage	Stage 1	Stage 2	Stage 3	Stage 4
				
Pentaradial symmetry	+	+	+	+
Primary podia	-	+	+	+
Adult skeleton	-	+	+	+
Body skeletal plates	-	-	+	+
Tube feet	-	-	+	+
Spines and/or spine primordia	-	-	-	+

[3,3',5-triiodothyronine] and thyroxine [T4] concentrations in larvae of two sea urchin species and one unicellular algal species that sea urchin larvae feed on (this was the apparent source for the thyroid hormones in the larvae) using radioimmunoassays after separation of individual thyroid hormones by HPLC. Suyemitsu et al. (1997) and Saito et al. (1998) showed that inhibitor (thiourea) treatment of non-feeding larvae of the sand dollar *Peronella japonica* resulted in lower T3 and T4 levels. Based upon these studies, we refer here to the active compounds, inhibited in our experiments by thiourea and rescuable by exogenous thyroxine, as thyroid hormone (TH).

We prepared thyroxine (T-1775 Sigma-Aldrich, St. Louis, MO) as described in Chino et al. (1994), and thiourea (a thyroxine synthesis inhibitor which acts by blocking iodine peroxidase activity; Sigma-Aldrich) in MFSW (millipore-filtered seawater; 0.45 μ m) at appropriate concentrations.

Experimental Designs

Dendraster excentricus larvae can be reared from 10°C to 24°C (Strathmann 1987) without affecting their relative developmental trajectories (McEdward 1985). Temperatures for the four experiments were as follows: morphometrics experiment: 11.5–14.0°C (30 June–12 August 2000; 42 days); plasticity experiment: 21–22°C (14–26 June 2002; 12 days); metamorphosis experiment 21–23°C (8 July–12 August 2000; 37 days); inhibitor experiment 18.8 \pm 1.3°C SD (30 June–12 August 2001; 42 days)].

To obtain gametes, we injected one male and one female (different ones in each experiment) with 0.55M KCl and set larval cultures up after hatching (as described in Strathmann 1987). The cultures were gently stirred using a motor-driven stirring apparatus (Strathmann 1987) or a shaker table, and we changed the water every two to three days. Unless stated otherwise, we set up larval cultures (at a maximal initial larval density of 1 larva/5 ml MFSW) at the stage when the invaginating echinus rudiment contacts the left hydrocoel, and fed larvae 6000 cells/ml *Dunaliella tertiolecta*. Larvae in the inhibitor experiment were starved when they had reached rudiment stage 3 (see Table 1).

Experimental Treatments

Morphometrics experiment: HIGH TH (10⁻⁹M thyroxine); LOW TH (10⁻¹¹M thyroxine); CONTROL; LOW INHIBITOR (10⁻⁴M thiourea); HIGH INHIBITOR (10⁻²M thiourea). Plasticity experiment: HFHT (6000cells/ml *D. tertiolecta*, 5 \times 10⁻¹⁰M thyroxine); LFHT (2000cells/ml *D. tertiolecta*, 5 \times 10⁻¹⁰M thyroxine); HFLT (6000cells/ml *D. tertiolecta*, 5 \times 10⁻¹¹M thyroxine); LFLT (2000cells/ml *D. tertiolecta*, 5 \times 10⁻¹¹M thyroxine); HF (6000cells/ml *D. tertiolecta*), LF (6000cells/ml *D. tertiolecta*). Metamorphosis experiment: HIGH TH (10⁻⁹M thyroxine); LOW TH (10⁻¹¹M thyroxine); CONTROL; INHIBITOR (1.67 \times 10⁻³M thiourea); MIXED (1.67 \times 10⁻³M thiourea plus 10⁻⁹M thyroxine). Inhibitor experiment (no food): TH (10⁻⁹M thyroxine);

CONTROL; INHIBITOR (10^{-3}M thiourea) and MIXED (10^{-3}M thiourea plus 10^{-9}M thyroxine).

Morphometric Measurements and Rudiment Stages

In the morphometrics experiment, we took measurements on five randomly chosen larvae (see Results for details) per independent replicate (three replicates in all experiments) after each water change. From captured images (using a Nikon [Tokyo, Japan] compound microscope with an attached Polaroid [Waltham, MA] video camera) we took the following morphometric measurements (as indicated in Figure 1A): postoral (PO) arm length, postdorsal (PD) arm length, body-midline length (BM), stomach length (SL), stomach width (SW), rudiment length (RL), and rudiment width (RW) using NIH Image (public domain; <http://rsb.info.nih.gov/nih-image>) software and our own macros written for NIH Image. We calculated stomach size (SS) and rudiment size (RS) as the square root of the cross-sectional area of an ellipsoid, using SW or RW and SL or RL as the ellipsoid axes respectively. For relative morphological character measurements we divided each absolute morphological character measurement by rudiment size. We consider PO, PD, and BM as larval characters, the stomach as both a larval and juvenile character since it is retained in the juvenile (Chia and Burke 1978), and the juvenile rudiment as a juvenile character. We also assigned each larva to developmental stages based on adult rudiment characters (Table 1; Fig. 1B), using images taken with differential interference (DIC) optics and/or polarized light.

For the plasticity experiment, we used a Nikon microscope E600 with a Nikon Coolpix 990 digital camera attached to it, which did not give clear views of the skeleton using polarized light. Therefore, we did measurements and staging as described above, except that we ultimately flattened each larva underneath a cover slide for rudiment stage analysis (see Table 1). We have since demonstrated that both techniques (polarized light in living larvae vs. flattening larvae) yield nearly identical results for rudiment staging in our hands (data not shown).

Metamorphic Competence and Settlement

We define settlement as the moment when tube feet stick out of the larva and it attaches firmly to the bottom of the culture dish. In the morphometrics experiment and the metamorphosis experiment, settlement occurred naturally (i.e., we added no settlement-inducing chemical). In the inhibitor and plasticity experiment, we induced settlement with 40mM excess KCl in MFSW. We define metamorphic competence as the time at which more than 30% of the larvae in a given replicate settled spontaneously or upon induction of settlement with KCl. Because the KCl response was so robust in the plasticity experiment, we did not use the 30% threshold but compared percent settlement in response to KCl directly. All juvenile measurements are test diameters (without spines).

Many observers have noted (e.g., Cameron and Hinegardner 1977) that echinoid larvae often have emergent tube feet well before settlement, and that such larvae can form non-permanent attachments to the substrate. Our experience,

though, is that a suction challenge to attached larvae distinguishes settled larvae from those merely sampling the substrate.

Different echinoid species differ in the extent to which they require a specific settlement cue or inducer. *Dendraster excentricus* larvae readily settle onto clean plastic or glass. Therefore, we feel justified in defining metamorphic competence here as either 30% spontaneous or 30% KCl-induced settlement. We recognize that such a broad definition for competence would not be applicable to those echinoid (or other) species that fail to settle so readily in the absence of a cue or inducer.

Statistics

We compared time to metamorphic competence, juvenile sizes, and larval growth among treatments as a function of rudiment stage using general linear model commands in SPSS (SPSS, Inc., Chicago, IL). We used a nested design to test for homogeneity of measurements among replicates. For the rudiment stage specific analysis and juvenile size, we used estimated marginal means for the comparison among treatments due to unequal number of replicate measurements (we present standard deviation instead of standard error in the relevant graphs). If necessary we used Bonferroni corrections for multiple comparisons. We analyzed larval mortality using Kolmogorov-Smirnov statistics.

RESULTS

Analysis of Thyroxine Effects on Larval and Juvenile Morphology

Comparison of age and rudiment stage specific analysis

We used the morphometrics experiment to compare measurements of larval and juvenile characters (Fig. 1) at age versus juvenile rudiment stage in the sand dollar *D. excentricus* (Fig. 2; see Table 1). In Figure 2 we used the method of Schlosser (2001) by plotting the age at which given rudiment stages were reached for the CONTROL (x-axis) against the age at which the comparable rudiment stages were reached (y-axis) in the presence of exogenous thyroid hormone (HIGH TH [10^{-9}M thyroxine]) and a thyroid hormone synthesis inhibitor (HIGH INHIBITOR [10^{-2}M thiourea]). Thus, larvae measured at the same time were not necessarily at the same developmental stage. Specifically, larvae in HIGH TH developed rudiment structures early; the reverse was true (although to a lesser degree) in HIGH INHIBITOR.

To undertake a meaningful comparison of relative investments into larval and juvenile structures by larvae reared under divergent food and chemical treatment conditions, we henceforth focus on comparisons among treatments grouped by juvenile rudiment stage (Table 2).

Analysis of larval, juvenile and stomach growth and development

Morphometrics experiment.—Figure 3 shows growth trajectories for each morphological character measurement (see Fig. 1) as a function of rudiment stage (see Table 1) for all experimental treatments (HIGH TH [10^{-9}M thyroxine], LOW

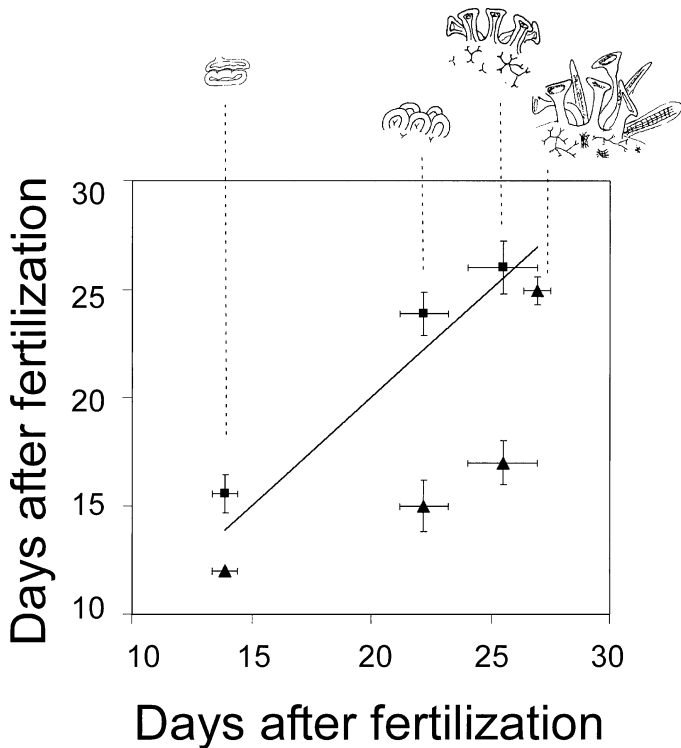


FIG. 2. Thyroid hormone (thyroxine) accelerates and a thyroid hormone synthesis inhibitor (thiourea) delays the developmental sequence of *Dendraster excentricus* larvae relative to the control (morphometrics experiment, approx. 13°C). Times to developmental stages 1–4 (drawn above the graph; see Table 1 and line drawings in this figure) for the control are plotted on the x-axis. Equivalent stages for thyroxine and thiourea are plotted on the y-axis. Points above the line indicate delayed development relative to the control; points below the line indicate accelerated development relative to the control. ▲ HIGH TH (10^{-9} M thyroxine) versus CONTROL. ■ HIGH INHIBITOR (10^{-2} M thiourea) versus CONTROL. Reference line indicates CONTROL versus CONTROL. Error bars represent one SE.

TH [10^{-11} M thyroxine], CONTROL, LOW INHIBITOR [10^{-4} M thiourea], and HIGH INHIBITOR [10^{-2} M thiourea]). A nested ANOVA revealed homogeneity for all measurements of morphological characters among replicates in each treatment ($P > 0.40$). Therefore, we pooled measurements of all replicates from the same treatment (for reference see Underwood 1997, p. 268). Table 2 summarizes the ANOVA results; we present the details of the ANOVA in the Appendix.

All three larval characters were significantly smaller in HIGH TH than in the CONTROL after rudiment stage 2 (Fig. 3A–C; Table 2; Appendix). For LOW TH, we detected no consistent trend (Fig. 3A,B; Table 2; Appendix). HIGH INHIBITOR larvae had significantly smaller PO and PD arm lengths at rudiment stages 1 and 2 (Fig. 3A,B; Table 2; Appendix). For LOW INHIBITOR, PO and PD arms were significantly shorter at rudiment stage 4 (Fig. 3A,B; Table 2; Appendix). Stomach size was significantly smaller in HIGH TH after rudiment stage 2 (Fig. 3D, Table 2; Appendix). For HIGH INHIBITOR and LOW INHIBITOR, stomach size was increased compared to the control (Fig. 3D, Table 2; Appendix). We detected no divergence in rudiment size be-

tween the control and any of the treatments after rudiment stage 1 (Fig. 3D; Table 2; Appendix).

We analyzed growth trajectories using a correlation analysis (Table 3) between each larval characteristic (PO, PD, BM; see Fig. 1A and below) or stomach size (SS) and rudiment size (RS; see Material and Methods). We used absolute rudiment size because it was the character least influenced by the experimental treatments and followed comparable growth trajectories in all treatments (Fig. 3E; Table 2; Appendix). For each independent replicate (beaker) we compared the Pearson correlation coefficients among treatments using an ANOVA with simple contrast. HIGH TH growth trajectories all had negative correlations, whereas HIGH INHIBITOR and the CONTROL had positive correlations, indicating that HIGH TH larvae allocated relatively more energy into juvenile rather than larval structures.

Phenotypic plasticity experiment and comparison with morphometrics experiment.—In Figure 4, we present the ratios of a given morphological character measurement over juvenile rudiment size for the morphometrics experiment and the phenotypic plasticity experiment. These ratios indicate the relative allocation of energy into growth and development of larval structures (PO, PD, BM) or the stomach versus juvenile rudiment structures (see also Strathmann et al. 1992). We only present data from rudiment stages 3 and 4 (see Table 1). Larvae allocate relatively more energy into growth and development of juvenile structures than into larval structures, when they are reared with either excess thyroid hormone (thyroxine) or high food (Fig. 4). The reverse is true when larvae are reared either with high levels of the thyroid hormone synthesis inhibitor thiourea (Fig. 4A) or with low food (Fig. 4B). The combined treatment of low levels of excess thyroid hormone plus low food (LFLT) resulted in a similar allocation ratio to high food (HF; Fig. 4b; see also Fig. 8).

In summary, exposure either to high levels of exogenous thyroid hormone or to high food levels resulted in an increased relative allocation of energy into the growth of juvenile structures and a reduced relative allocation into the growth of larval structures (see Table 3). The investment profile in low food-reared larvae (increased investment into larval rather than juvenile structures) was reversed with the addition of low levels of exogenous thyroid hormone. Together, these results strongly suggest a connection between the morphometric effects of food and thyroid hormone on larval and juvenile growth in echinoid larvae.

Metamorphic Competence and Juvenile Size

We present the timing of settlement (see Materials and Methods) for all treatments from the metamorphosis experiment in Fig. 5A. CONTROL *D. excentricus* larvae reached metamorphic competence 18.3 ± 1.8 (1 SE of mean) days after fertilization. Competence was reached in HIGH TH (10^{-9} M thyroxine) larvae in less than half that time (7.7 ± 0.67 days after fertilization); and was accelerated in LOW TH (10^{-11} M thyroxine) as well (14.3 ± 0.67 days after fertilization). An ANOVA with post-hoc comparison using Bonferroni corrections revealed a significant difference between HIGH TH and CONTROL ($P = 0.003$), but not between LOW TH and CONTROL ($P = 0.40$). Larvae from INHIBITOR

TABLE 2. Qualitative summary of ANOVA results for morphological character measurements as a function of rudiment stage for all experimental treatments (morphometrics experiment, approx. 13°C) compared to CONTROL (see Table 1 for our definitions of juvenile rudiment stages). ↓ indicates that the morphological character measurement was significantly ($P < 0.05$) smaller than in CONTROL. ↑ indicates that the morphological character measurement was significantly ($P < 0.05$) larger than in CONTROL. N/A, not available.

Character	Rudiment stage	Treatment			
		HIGH TH (10^{-9} M T4)	LOW TH (10^{-11} M T4)	LOW INHIBITOR (10^{-4} M thiourea)	HIGH INHIBITOR (10^{-2} M thiourea)
Postoral arm length	1			↓	↓
	2	↓		↓	↓
	3	↓			
	4	↓		↓	N/A
Postdorsal arm length	1			↓	↓
	2	↓	↑		↓
	3				
	4	↓		↓	N/A
Body midline length	1		↓	↓	
	2	↓			
	3	↓			
	4	↓			N/A
Stomach size	1				↑
	2	↓			↑
	3	↓			↑
	4	↓			N/A
Rudiment size	1	↑		↓	
	2				
	3				
	4				N/A

(1.67×10^{-3} M thiourea) did not reach the 30% settlement threshold over the course of the experiment. This thiourea-induced delay was rescued in the MIXED treatment (10^{-9} M thyroxine plus 1.67×10^{-3} M thiourea), where 30% settlement was reached after 12.3 ± 1.8 days. Note that settlement was most synchronous in HIGH TH (Fig. 5A).

In the plasticity experiment, we found that food also had significant effects on timing to metamorphic competence in *D. excentricus* larvae (Fig. 6A), as has been previously reported (Hart and Strathmann 1994). Under high food conditions (HF), larvae settled earlier than in low food (LF; Fig. 6A). In the two treatments in which we combined food with high thyroid hormone concentrations (HFHT, LFHT), the larvae settled sooner than in any of the other treatments (Fig. 6A). The timing of settlement was similar in LFLT and HF, indicating that hormones can mimic the effect of food.

In addition, we report a significant decrease in juvenile size (test diameter) at settlement in HIGH TH ($162.50 \pm 30.10 \mu\text{m}$; $n = 56$; $P < 0.001$) and MIXED ($227.94 \pm 48.84 \mu\text{m}$; $n = 10$; $P < 0.001$) relative to the CONTROL ($290 \pm 21.31 \mu\text{m}$; $n = 9$) in the metamorphosis experiment. We found no significant difference between CONTROL and LOW TH ($281.56 \pm 45.91 \mu\text{m}$; $n = 11$; $P = 0.585$). Note that these differences in juvenile size (Fig. 5B) could be explained by differences in time to settlement (Fig. 5A).

In the plasticity experiment, the results with juvenile size (Fig. 6b) are comparable to the morphometrics experiment (data not shown) and the metamorphosis experiment (above). In general, juveniles from the high thyroid hormone (thyroxine) treatments (HFHT, LFHT) were significantly smaller than juveniles from most other treatments. In the low and no hormone treatments (HFLT, LFLT, HF, LF), LF, and LFLT, juveniles were significantly smaller than HF juveniles. How-

ever, addition of low levels of thyroid hormone to the low food treatment (LFLT) did not change juvenile size when compared to LF.

In summary, food and hormone both lead to accelerated growth to metamorphosis, and a combined low hormone and low food treatment mimics the acceleratory effects of high food. Taken together, these results indicate a connection between the metamorphic effects of food and thyroid hormone in echinoid larvae. Still, although increased food levels lead to settlement at an increased juvenile size (Hart and Strathmann 1994; this study), high levels of thyroid hormone lead to settlement at a reduced size when compared to appropriate controls.

Endogenous Hormone Synthesis

To test whether thiourea effects on *D. excentricus* larvae were direct or indirect (via thiourea effects on algae) we transferred rudiment stage 3 *D. excentricus* larvae (see Table 1) to one of four treatments in the absence of food (i.e. no algae): 10^{-9} M thyroxine (TH); CONTROL; 10^{-3} M thiourea (INHIBITOR); 10^{-3} M thiourea plus 10^{-9} M thyroxine (MIXED). We detected a subtle but significant delay in settlement in INHIBITOR ($26.33 \pm 2.52\%$ SE settled on days 14–18) when compared with the CONTROL ($37.33 \pm 4.59\%$; two-tailed t -test: $t_{28, -2.099} < 0.05$; Fig. 7). TH larvae settled much earlier than in the CONTROL ($80 \pm 5.77\%$ by day 10). In the MIXED treatment, the thiourea-induced settlement delay was rescued with exogenous TH ($66.67 \pm 12.02\%$ by day 10). These results indicate that *D. excentricus* larvae can synthesize thyroid hormone (or a thyroid hormone-like compound; but see Materials and Methods) endogenously. However, this endogenous thyroid hormone production is not suf-

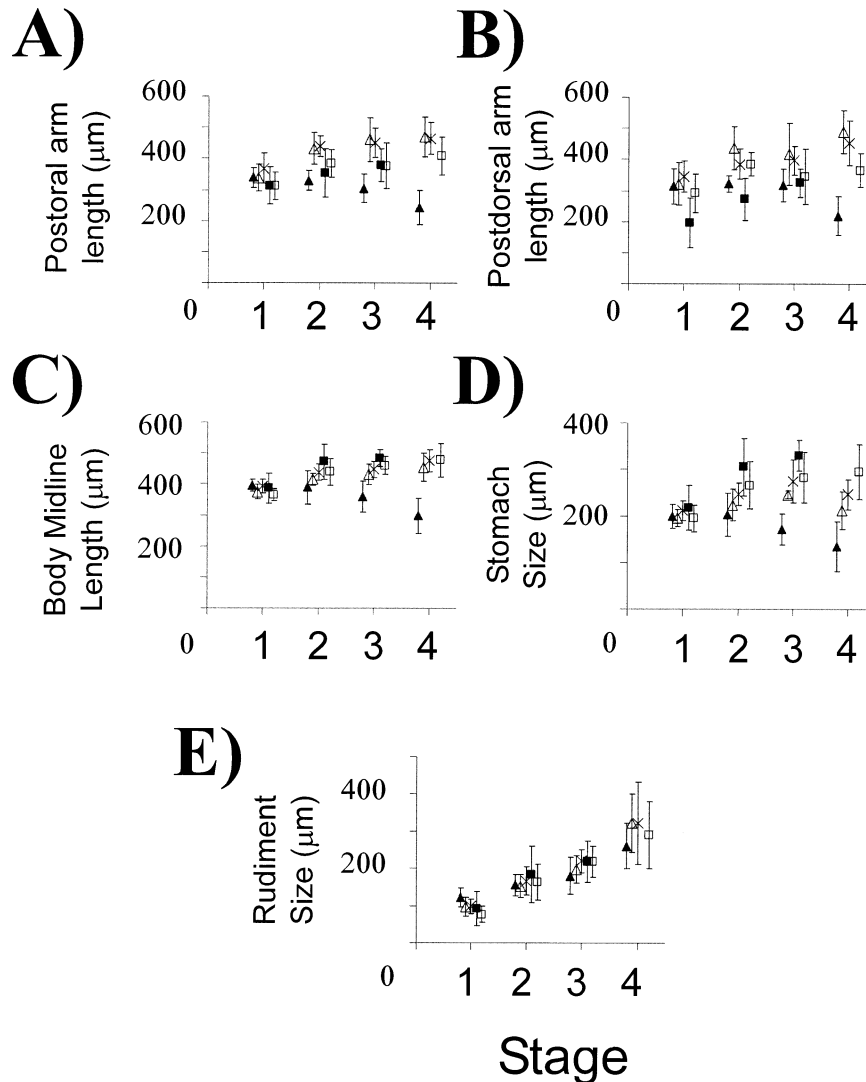


FIG. 3. Heterochronic changes in relative allocation to larval and juvenile structures as a result of thyroid hormone (thyroxine) and thyroid hormone synthesis inhibitor (thiourea) treatments. Growth curves for larval characters in μm (postoral arm length [PO], postdorsal arm length [PD], body midline length [BM], stomach size (SS), and juvenile rudiment size (RS) as a function of juvenile rudiment stage for all four experimental treatments and the control at 13°C (morphometrics experiment; see Figure 1A for a guide to structures; see Table 1 for rudiment stage criteria; see Materials and Methods for our method for calculating SS and RS). Measurement sets were taken at 12, 16, 23, and 28 days after fertilization. Error bars indicate \pm one SD (SD is used here due to unequal sample size after staging of larval development). \blacktriangle HIGH TH (10^{-9}M thyroxine); \triangle LOW TH (10^{-11}M thyroxine); \times CONTROL; \square LOW INHIBITOR (10^{-4}M thiourea); \blacksquare HIGH INHIBITOR (10^{-2}M thiourea).

ficient to allow *D. excentricus* larvae to reach metamorphic competence in the complete absence of larval food; such larvae do not develop beyond the appearance of pentaradial symmetry in the rudiment (rudiment stage 1, see Table 1; data not shown).

Furthermore, the finding that these inhibitor effects are rescued by the addition of excess thyroxine indicates that thiourea is an effective, specific blocker of thyroid hormone production and/or signaling in *D. excentricus* larvae.

Mortality and Abnormalities

We detected no significant difference in mortality between any of the treatments and the control using a two-sample Kolmogorov-Smirnov test in the morphometrics experiment

(less than 11.2% average daily mortality in all treatments; note that “mortality” also includes any larvae lost during transfer) the plasticity experiment (less than 4.4% average daily mortalities) and the metamorphosis experiment (less than 6.5% in all treatments). We also detected no significant difference in abnormality in the morphometrics experiment between any of the treatments and the control using a two-sample Kolmogorov-Smirnov test (less than a 5.8% average daily occurrence in all treatments) and comparable levels of abnormality were observed in the plasticity experiment.

Growth curves of all morphological characters and their statistical analyses are included as supplementary material (Supplement 1 and Supplement 2, respectively; available online at <http://dx.doi.org/10.1554/03-243.1.s1> and <http://dx.doi.org/10.1554/03-243.1.s2>).

TABLE 3. Analysis and comparison of growth trajectories between the HIGH TH (10^{-9} M thyroxine), CONTROL, and HIGH INHIBITOR (10^{-3} M thiourea) and for each morphological character measurement relative to the rudiment size (morphometrics experiment, approx. 13°C). For each independent replicate, the Pearson correlation coefficient between the morphological character measurement and the rudiment size was analyzed and compared between the treatments and the control using ANOVA with simple contrast. Mean \pm 1 SE (P -value).

Character	Treatment		
	HIGH TH	CONTROL	HIGH INHIBITOR
Postoral arm length versus rudiment size	$-0.65 \pm 0.01^*$ ($P < 0.01$)	0.52 ± 0.12	0.60 ± 0.11 ($P = 0.56$)
Postdorsal arm length versus rudiment size	$-0.68 \pm 0.01^*$ ($P < 0.01$)	0.57 ± 0.11	0.59 ± 0.03 ($P = 0.82$)
Body midline length versus rudiment size	$-0.63 \pm 0.07^*$ ($P < 0.01$)	$0.78 \pm 3.06 \times 10^{-2}$	0.90 ± 0.07 ($P = 0.10$)
Stomach size versus rudiment size	$-0.49 \pm 0.18^*$ ($P < 0.01$)	0.45 ± 0.20	0.82 ± 0.05 ($P = 0.14$)

* $P < 0.05$.

DISCUSSION

Although complex, metamorphic life histories have evolved independently in a wide variety of animal phyla, many of these life-cycles appear to have converged upon a common mechanism: the life cycle transitions are controlled by hormones (reviewed in Matsuda 1987). The best-studied cases involve the derived, metamorphic life histories of holometabolous insects (bees, beetles, butterflies, etc.) and amphibians (frogs, salamanders, etc.), which are orchestrated, respectively, by steroid and thyroid hormones (reviewed in Nijhout 1999; Rose 1999). Furthermore, the evolution of novel life histories within these two disparate animal groups appears to involve alterations in the regulation of these hormones. For example, larval reproduction in flies (Hodin and Riddiford 2000), direct development in frogs (Hanken et al. 1997; Jennings and Hanken 1998; Callery and Elinson 2000), and neoteny in salamanders (Frieden 1981; Kühn and Jacobs 1989; Yaoita and Brown 1990; Galton 1992) all entail major alterations in the timing of hormonal release and/or the cellular hormonal response. Although little information is available about metamorphic roles of hormones in most other phyla, a striking case of parallel evolution has recently been reported (see below) in amphibians and echinoderms (sea urchins, starfish and their kin): both groups use thyroid hormone as a regulator for metamorphosis (defined here broadly to include the lengthy development of the juvenile rudiment in echinoderms). Whether thyroid hormones are involved in the evolution of novel life histories in echinoderms, as they are in amphibians, has not been previously explored.

As is true in amphibians, several planktotrophic sea urchins (Chino et al. 1994; Johnson 1998), two sand dollar species (Suyemitsu et al. 1997; Saito et al. 1998; Hodin et al. 2001), and a starfish (Johnson and Cartwright 1996) complete metamorphosis early in the presence of exogenous thyroxine (T₄), one form of thyroid hormone. Intriguingly, the major source of the hormone for feeding echinoderm larvae appears to be the planktonic algae that they consume (Chino et al. 1994). We propose that thyroid hormones are an environmental signal that larvae use as an indicator of nutritional state (for a similar case in amphibians, see Pfennig 1992). We will discuss this hypothesis below in the context of phenotypic plasticity, juvenile size, and life-history evolution in echinoids.

Thyroid Hormone as a Cue for Adaptive Phenotypic Plasticity in Echinoid Larvae

Phenotypic plasticity is ontogenetic variability within a genotype due to naturally occurring or experimentally imposed environmental heterogeneity (reviewed in Schlichting and Pigliucci 1998). An adaptively plastic response depends upon the ability of an organism to sense an environmental cue (such as day length, temperature, etc.), allowing it to respond appropriately to changing conditions (reviewed in Schlichting and Pigliucci 1998). Larval food concentrations in the marine environment undergo large temporal and spatial fluctuations (Pechenik 1987; Fenaux et al. 1994; Morgan 1995). Several laboratory and field studies on echinoid larvae (Strathmann et al. 1992; Fenaux et al. 1994; Hart and Strathmann 1994) have demonstrated that when food is limited, a shift occurs toward investment into growth and development of larval rather than juvenile structures, resulting in an increase in feeding efficiency. When larvae live in an enhanced food environment, by contrast, investment into larval structures is reduced and the juvenile rudiment develops at a faster rate, an apparently adaptive response to avoiding the dangers inherent in the plankton (reviewed in Morgan 1995).

Here we report differential investment into juvenile versus larval structures in *D. excentricus* larvae that were exposed to different levels of exogenous thyroid hormones (specifically thyroxine) in either low or high food conditions. Excess amounts of exogenous thyroid hormone (TH) induce a morphometric and metamorphic response in *D. excentricus* larvae similar to the effect of excess food (Fig. 8). Indeed, *D. excentricus* larvae exposed simultaneously to low food and low TH levels resemble *D. excentricus* larvae exposed to high food levels in the absence of excess TH. Furthermore, we show that larvae reared with the TH synthesis inhibitor thiourea resemble larvae raised in reduced food conditions. These similar developmental responses lead us to conclude that echinoid larvae use TH levels as an indicator of food availability in the plankton (see Thyroid Hormones As an Indicator of Larval Nutrition, below). This notion that exogenous TH can induce an adaptively plastic morphometric response similar to the effects of diet is not without precedent. Pfennig (1992) showed that TH (thyroxine) treatment of spadefoot

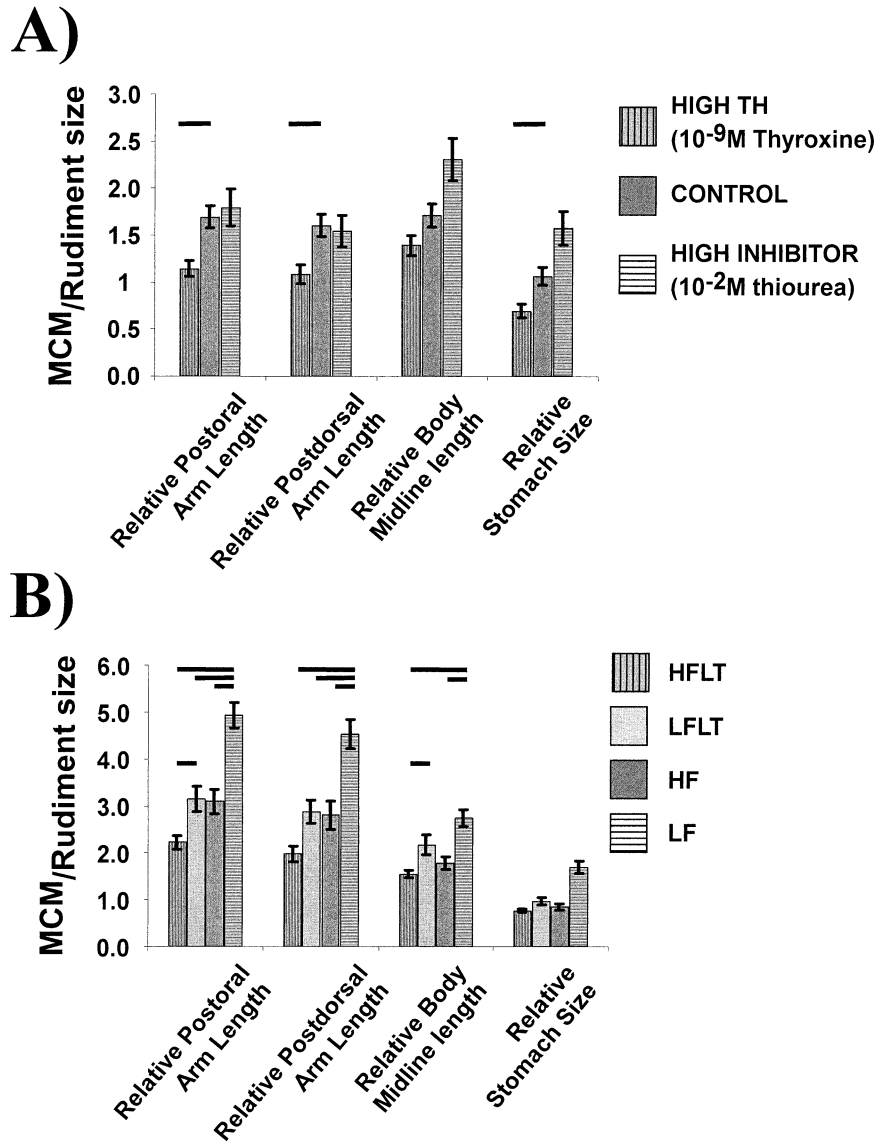


FIG. 4. Thyroid hormone (thyroxine) and food have similar effects on the relative investment into larval versus juvenile growth in *Dendraster excentricus* larvae. Morphometric analysis from morphometrics experiment (A) and plasticity experiment (B) at rudiment stage 3 and 4 combined (see Table 1). Relative morphometric character measurements (relative postoral arm length, relative postdorsal arm length, relative body midline length and relative stomach size) are calculated by dividing the appropriate morphological character measurement (MCM) by rudiment size (see Materials and Methods and Figure 1). All values are means \pm one SE. Horizontal bars indicate significant pair wise differences between mean values based on multivariate analysis of variance using Bonferroni corrections for multiple comparisons. Morphometrics experiment: HIGH TH (10^{-9} M thyroxine); CONTROL; HIGH INHIBITOR (10^{-2} M thiourea). Plasticity experiment: HFLT (6000 cells/ml *Dunaliella tertiolecta*; 5×10^{-11} M thyroxine); LFLT (2000 cells/ml *D. tertiolecta*; 5×10^{-11} M thyroxine); HF (6000 cells/ml *D. tertiolecta*); LF (2000cells/ml *D. tertiolecta*).

toad tadpoles (*Scaphiopus multiplicatus*) induces a carnivorous larval morph, a response also induced by a diet switch to shrimp (known to contain T2—a form of TH) from a commercial omnivorous fish food.

We propose that in echinoderm larvae, TH acts in a signaling system that activates juvenile development while repressing larval growth. To further test this hypothesis, we can examine the dose response of starved larvae to TH, to see whether TH alone can produce ontogenetic effects similar to ingested food. We can also pretreat algae with TH synthesis

inhibitors to try to produce well-fed larvae that respond as if they are being starved.

Although the plastic response of echinoderm larvae to environmental food levels has been suggested to be adaptive, no study to date (including our own) has directly addressed the fitness consequences in juveniles of this induced plastic response. Hart and Strathmann (1994) noted that direct fitness comparisons between larvae with the long-armed (delayed metamorphosis) and short-armed (accelerated metamorphosis) phenotypes suffer from the confounding effect of food

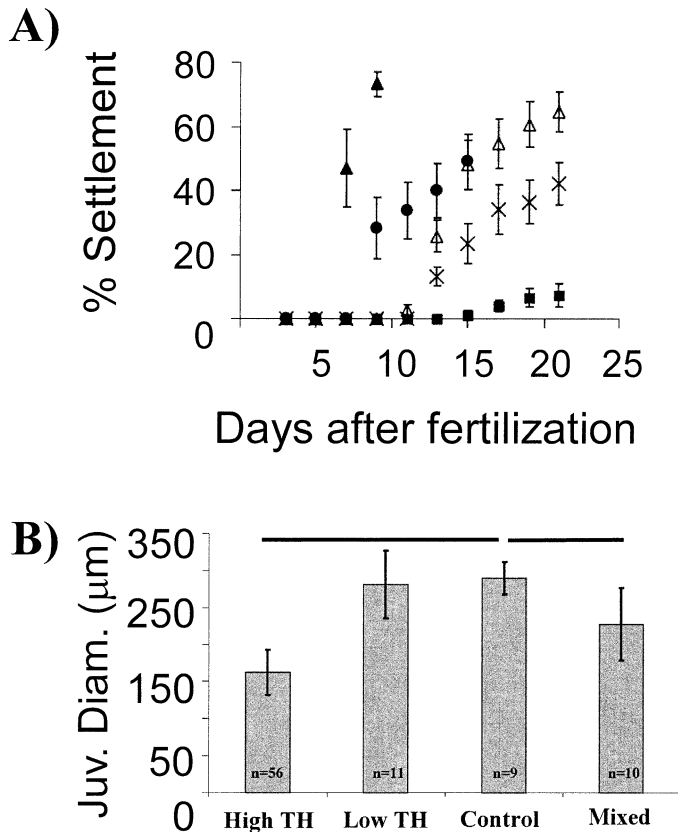


FIG. 5. Exogenously applied thyroid hormone (thyroxine) results in earlier settlement of *Dendraster excentricus* larvae at a reduced juvenile size; the thyroid hormone synthesis inhibitor thiourea delays settlement (metamorphosis experiment, approx. 22°C). (A) Percent settlement over time for all four experimental treatments and the control (error bars represent one SE). Note that in the MIXED treatment, the thiourea-induced delay of settlement is rescued by thyroxine. ▲ HIGH TH (10^{-9} M thyroxine); △ LOW TH (10^{-11} M thyroxine); × CONTROL; ■ INHIBITOR (1.6×10^{-3} M thiourea); ● MIXED (10^{-9} M thyroxine plus 1.6×10^{-3} M thiourea). (B) Juvenile (test) diameters (Juv. Diam.) at settlement for the four experimental treatments and the control. We only measured diameters for juveniles after settlement was greater than 30% in a given replicate (see Materials and Methods). Error bars represent one SD; *n*, number of measurements per treatment. Similar results are seen at 13°C (not shown).

treatment. These authors proposed to uncouple the plastic response from the food effects by generating larvae with the high food (short-armed) morphology at a low food level (using TH), and the reverse (using inhibitors). Due to the confounding effects of inhibitors on the algae themselves (not shown, but described below), the latter experiment is unfortunately not feasible. As for the use of TH, since it also results in precocious settlement at a small size (via accelerated rudiment growth), its usefulness in testing the specific adaptive hypothesis proposed by Hart and Strathmann (1994) is also questionable. Still, in our plasticity experiment, we identified what may be an ideal protocol for a fitness comparison akin to the one that Hart and Strathmann proposed. Our analysis of the low food (LF) and low food/low hormone (LFLT) treatments revealed that the latter developed to metamorphosis faster via the short-armed (high food-like) phenotype,

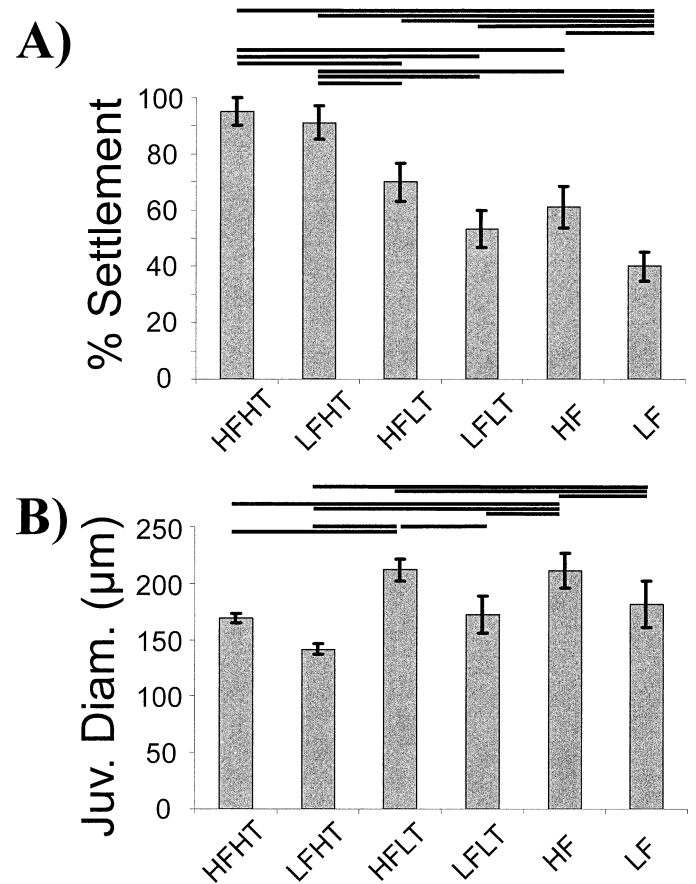


FIG. 6. Both excess food and thyroid hormone both accelerate development to metamorphosis; low food and thyroid hormone lead to settlement at a reduced juvenile size. Metamorphosis (A) and juvenile size (measured as juvenile diameter, Juv. Diam.) (B) in the plasticity experiment. Mean values \pm one SE. Horizontal bars indicate significant difference between mean values ($P < 0.05$) using univariate analysis of variance with Bonferroni corrections for multiple comparisons. HFHT (6000cells/ml *Dunaliella tertiolecta*; 5×10^{-10} M thyroxine), LFHT (2000cells/ml *D. tertiolecta*; 5×10^{-10} M thyroxine), HFLT (6000cells/ml *D. tertiolecta*; 5×10^{-11} M thyroxine), LFLT (2000cells/ml *D. tertiolecta*; 5×10^{-11} M thyroxine), HF (6000cells/ml *D. tertiolecta*), LF (2000cells/ml *D. tertiolecta*). HFHT ($n = 4$), LFHT ($n = 6$), HFLT ($n = 6$), LFLT ($n = 6$), HF ($n = 6$), LF ($n = 6$). *n*, numbers of replicate treatments; five individuals/replicate.

with no ultimate difference in juvenile size (but with presumed differences in stored nutrition; see below). The consequence of feeding efficiency on fitness can now be tested by directly comparing postlarval performance in juveniles derived from the LF and LFLT treatments. We predict that LF-derived juveniles would grow more quickly after settlement than LFLT-derived juveniles, indicating a selective advantage to the low food (long-armed, delayed-settlement) phenotype.

Differences between Thyroid Hormone and Food Effects: Stomach Size

We found that stomach size is differently affected by food and hormone treatments. Several food limitation experi-

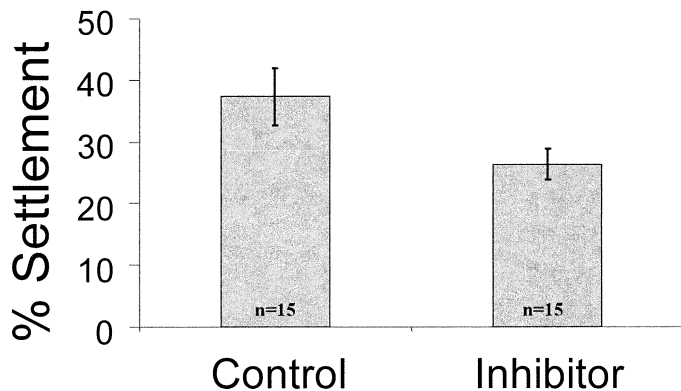


FIG. 7. Thiourea delays settlement in the absence of food in *Dendraster excentricus* larvae. This suggests that *D. excentricus* larvae can synthesize thyroid hormones endogenously. Larvae were starved after rudiment stage 3 (see Table 1) and exposed to different treatments (inhibitor experiment, approx. 19°C). Relative to the starved control, settlement occurred earlier when 10^{-9} M thyroxine (TH; see text) was present and settlement was delayed when larvae were treated with 10^{-3} M thiourea (INHIBITOR). However, this effect was rescued with a mixed treatment: 10^{-9} M thyroxine plus 10^{-3} M thiourea (MIXED; see text). Error bars represent one SE.

ments, including our own, indicate that larvae from low food treatments have shrunken stomachs, whereas their well-fed counterparts have enlarged stomachs. By contrast, our TH-treated *D. excentricus* larvae had reduced stomachs, whereas inhibitor-treated larvae had enlarged stomachs relative to controls, and these effects were dose-responsive (note, however, that it is only our measurements of absolute stomach size [not shown] that show this different pattern between food and hormone; when calculated relative to rudiment size, the effects of food and hormone are quite similar; see Fig. 4).

Unlike the larval arms and the juvenile rudiment, which are larval- and juvenile-specific structures respectively, the larval stomach in echinoids also acts as a nutritional storage organ, and is inherited by the juvenile (Chia and Burke 1978). Since TH-treated larvae have reduced arm growth, they presumably have lower feeding rates than do control larvae (Hart and Strathmann 1994). Still, such larvae invest more energy into juvenile rudiment development than controls, which must come at the expense of nutritional storage in and growth of the stomach. Thiourea-treated larvae, by contrast, delay juvenile rudiment development, and therefore can be hypothesized to invest more of their nutritional intake into stomach storage and growth.

Thyroid Hormones As an Indicator of Larval Nutrition: Implications for Juvenile Size in Echinoids

Many marine invertebrates have a complex life history, often involving a feeding planktonic larval stage, which has an entirely distinct morphology, behavior, and habitat from the benthic adult. Although some progress has been made in recent years in identifying possible settlement cues (reviewed in Hadfield 2000), very little is known about the mechanisms by which larvae attain the competence to respond to such settlement cues. In some insects, developmental transitions (molting and metamorphosis) have been shown to involve attainment of a critical size, and that structures such as stretch

receptors in the cuticle are involved in sensing body size (reviewed in Nijhout 1994, 1999).

TH levels might be a crucial component of the signal for metamorphic competence in echinoids and possibly other echinoderms as well. We infer from our results, as well as those of Chino et al. (1994) and Hodin et al. (2001), the following mechanistic scenario. The TH that is present in planktonic algae accumulates in the larval body during feeding. During this period, the accumulating TH is directing the growth and development of the juvenile rudiment. Then, competence is reached after internal TH levels reach a particular threshold and the larva becomes responsive to specific settlement cues. By exposing larvae of *D. excentricus* to excess exogenous TH at different food concentrations, competence was reached earlier than in the corresponding controls. However, the shorter larval period of experimental larvae resulted in the acquisition of less total nutrition than in control larvae, and earlier metamorphic competence was attained by a significantly accelerated development of the juvenile rudiment. As a consequence, juveniles from the experimental high TH treatment, for example, metamorphosed at about half the normal size (juvenile test diameter). This degree of juvenile size reduction is unprecedented for planktotrophic echinoids reared under any condition. Moreover, these results suggest that larval TH levels, rather than juvenile size itself, is a critical indicator of metamorphic competence.

Our unpublished data (with A. Reitzel) for the sand dollar *Leodia sexiesperforata* further supports this suggestion that competence is connected to larval TH levels. *Leodia sexiesperforata* larvae develop from eggs with approximately six times the energy content of *D. excentricus* eggs, but still need to feed in order to reach competence (L.R. McEdward lab, unpubl. data). However, when we treated starved *L. sexiesperforata* larvae with TH, they completed metamorphosis and formed living juveniles (A. Heyland, A. Reitzel, and J. Hodin, unpubl. ms.). These data suggest not only that TH in the absence of exogenous food is sufficient to induce development through metamorphosis in *L. sexiesperforata*, but also that TH levels are somehow connected to the attainment of metamorphic competence.

The length of the larval period and juvenile size are two traits that can potentially shape the evolution of life-history strategies in marine invertebrates. Due to high mortality in plankton, reduction of the planktonic period should generally be advantageous (Rumrill 1990; for review see Morgan 1995). However, a reduced larval period could lead to a reduced juvenile size (or a reduction in stored energy, as discussed above), which might have negative fitness consequences. Although such fitness consequences have been previously discussed (Strathmann 1974, 1977; Kolding and Fenchel 1981; Perron 1986; Emler et al. 1987; Pechenik et al. 1998), experimental tests are relatively rare (but see Emler and Hoegh-Guldberg 1997; Gosselin and Qian 1997). Our results demonstrate for the first time that metamorphosis at a substantially reduced juvenile size is mechanistically possible in planktotrophic echinoid larvae (for data on lecithotrophic larvae see Okazaki and Dan 1954; Emler and Hoegh-Guldberg 1997). This finding lends support to the notion that such juveniles would suffer reduced fitness, possibly by lowering their competitive ability and/or increasing their vul-

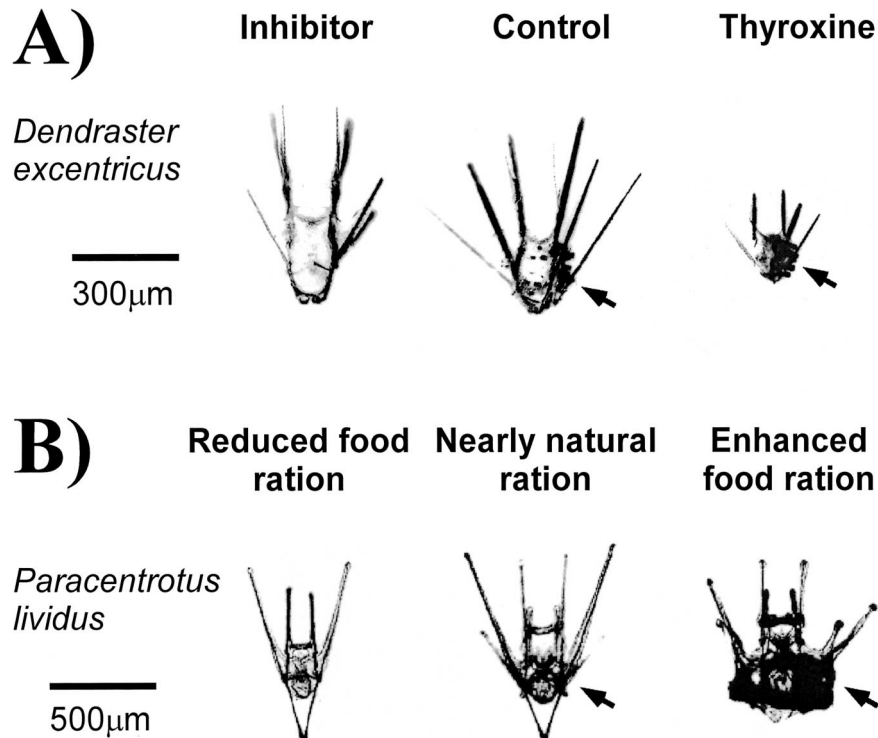


FIG. 8. Similar morphological effects of thyroid hormone and food on echinoid pluteus larvae. (A) Morphological effects of thyroid hormone (10^{-9} M thyroxine; HIGH TH) and a thyroid hormone synthesis inhibitor (10^{-2} M thiourea; HIGH INHIBITOR) on *Dendraster excentricus* larvae (23 days after fertilization at 13°C ; morphometrics experiment). (B) Morphological effects of reduced (left), nearly natural (middle), and enhanced (right) natural plankton on larvae of the sea urchin *Paracentrotus lividus* Lamarck (11 days after fertilization at approx. 20°C ; pictures in B reproduced from Strathmann et al. 1992, pp. 976–977). Arrows indicate the accelerated juvenile rudiments in the thyroid hormone-treated (A, right panel) and enhanced-food (B, Right panel) larvae when compared to the control (A, middle panel) and nearly-natural-food (B, middle panel) larvae, respectively. Juvenile rudiments are not visible at this stage in inhibitor-treated (A, left panel) and reduced-food (B, left panel) larvae.

nerability to predation. Such hypotheses can now be tested using TH as an experimental tool.

Our conclusion that echinoid larvae use TH levels as an indicator of larval nutrition depends upon the finding that the production of TH-like compounds is widespread among disparate algal taxa (Tsuchiya and Ito, reported in Tsuchiya 1969; Ragan 1981; Chino et al. 1994; reviewed in Eales 1997). Production of such a compound has not been reported for *Dunaliella tertiolecta*, the unicellular green alga that we used as larval food in this study. However, our unpublished results with the TH synthesis inhibitor thiourea demonstrated a reversible, dose-responsive effect of this inhibitor on *D. tertiolecta* growth rates, providing strong indirect evidence that this alga produces TH-like compounds. A greater diversity of phytoplankton species should be investigated for their relative TH contents, since these would represent the naturally occurring sources of hormones available to planktotrophic larvae such as *D. excentricus*.

Thyroid Hormones and Life-History Evolution in Echinoderms

One of the most striking life-history patterns in marine invertebrates in general, and echinoderms specifically, is that similar-looking adults can be produced from radically different ontogenetic routes: feeding (planktotrophic) development from relatively small eggs, or nonfeeding (lecitho-

trophic) development from large eggs via entirely dissimilar larvae (reviewed in Strathmann 1985). Although this life-history diversity seems to indicate a substantial flexibility of development, further analysis suggests that there might be constraints on the evolution of lecithotrophy from planktotrophic ancestors (see also Hodin et al. 2001).

Lecithotrophic taxa are found in all five extant echinoderm classes, with the class Crinoidea (sea lilies and feather stars) being entirely lecithotrophic. In the four other echinoderm classes, numerous evolutionary transitions from planktotrophy to lecithotrophy have been hypothesized (reviewed in Wray 1996). Still, the taxonomic distribution of lecithotrophs within these classes appears decidedly nonrandom. In the echinoids, for example, most of the 20 or so (Emler 1990, 1995; Wray 1996; G. A. Wray, pers. comm. 2001) independently evolved instances of lecithotrophy are concentrated in a few orders, notably the orders Clypeasteroidea (sand dollars and sea biscuits), Spatangoida (heart urchins) and Cidaroida (pencil urchins). By contrast, two of the largest and most diverse orders of regular sea urchins, the orders Diadematoidea and Echinoidea, have no and relatively few independently evolved lecithotrophs, respectively (reviewed in McEdward and Miner 2001).

We suggest that one factor that might explain such a non-random distribution of lecithotrophs is differences among taxa in the ability of their larvae to synthesize TH endoge-

nously. We hypothesize that endogenous synthesis of TH is a necessary preadaptation for the evolution of lecithotrophy. Those taxa that plesiomorphically have the ability to synthesize this hormone endogenously would be expected, therefore, to undergo parallel evolutionary transitions from planktotrophy to lecithotrophy more frequently.

Endogenous synthesis of TH has been reported for one lecithotrophic echinoderm, the sand dollar (order Clypeasteroidea) *Peronella japonica* (Suyemitsu et al. 1997; Saito et al. 1998). This, along with our data on the planktotrophic sand dollar *D. excentricus* (this study) and the facultative feeding sea biscuit *Clypeaster rosaceus* (A. Heyland and A. Reitzel, unpubl. data), suggests that clypeasteroid larvae have the capacity (albeit limited in the case of *Dendraster*) for endogenous TH synthesis. By contrast, two planktotrophic sea urchin species from the order Echinoidea (which has relatively few lecithotrophic taxa) do not show obvious inhibitor effects, suggesting that they produce little if any TH endogenously (Chino et al. 1994). These data support our hypothesis that endogenous TH production can be considered a preadaptation for the evolution of lecithotrophy.

A broad taxonomic survey of lecithotrophs and their planktotrophic relatives (using an approach that we took here for the inhibitor experiment) should reveal differing effects of TH synthesis inhibitors on juvenile morphogenesis and development to metamorphic competence. We predict that planktotrophic species from the orders Clypeasteroidea, Cidaroida, and Spatangoida will consistently show more pronounced inhibitor effects than their counterparts in the orders Diadematoidea and Echinoidea. Our recent unpublished data (in collaboration with T. Caprio) with *Diadema antillarum* larvae provides some support for this prediction.

The above scenario begs the following question: if ability to synthesize TH endogenously first evolved before the origin of lecithotrophy in these groups, then what could be a selective advantage of internal hormone synthesis itself? One possible advantage could be to modify the phenotypically plastic response that larvae have to ingested TH. Such larvae might exist in a relatively predictable planktonic environment, such that there is no adaptive value to maintaining a great degree of plasticity. The internal production of TH could dampen the plastic effects of temporary food fluctuations in the environment, as well as those that we see in the laboratory. Furthermore, planktotrophic echinoderm larvae developing from relatively large eggs could undergo metamorphosis more quickly, thereby escaping the dangerous planktonic environment (Rumrill 1990; see Morgan 1995) *only* if they do not depend upon feeding in the plankton to obtain all of the required TH. These hypotheses make a testable prediction: the ability to synthesize TH endogenously is inversely proportional to the scope of plasticity, and directly proportional to egg size in a given taxon. Experiments with clypeasteroid species representing a tenfold range in egg volume (*D. excentricus*, *C. rosaceus*, and *P. japonica*) offer initial support for this prediction. Addressing this hypothesis further could provide the synthesis of ecological and ontogenetic mechanisms necessary to account for any such patterns in life-history evolution.

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LITERATURE CITED

- Bertram, D. F., and R. R. Strathmann. 1998. Effects of maternal and larval nutrition on growth and form of planktotrophic larvae. *Ecology* 79:315–327.
- Boidron-Metairon, I. F. 1988. Morphological plasticity in laboratory-reared echinoplutei of *Dendraster excentricus* (Eschscholtz) and *Lytechinus variegatus* (Lamarck) in response to food conditions. *J. Exp. Mar. Biol. Ecol.* 119:31–41.
- Callery, E. M., and R. P. Elinson. 2000. Thyroid hormone-dependent metamorphosis in a direct developing frog. *Proc. Natl. Acad. Sci. USA* 97:2615–2620.
- Cameron, R. A., and R. T. Hinegardner. 1974. Initiation of metamorphosis in laboratory cultured sea urchins. *Biol. Bull.* 146:335–342.
- Chia, F. S., and R. D. Burke. 1978. Echinoderm metamorphosis: Fate of larval structures. Pp. 219–234 in F. S. Chia and M. E. Rice, eds. *Settlement and metamorphosis of marine invertebrate larvae*. Elsevier, New York.
- Chino, Y., M. Saito, K. Yamasu, K. Suyemitsu, and K. Ishihara. 1994. Formation of the adult rudiment of sea urchins is influenced by thyroid-hormones. *Dev. Biol.* 161:1–11.
- Christiansen, F. B., and T. M. Fenchel. 1979. Evolution of marine invertebrate reproductive patterns. *Theor. Popul. Biol.* 16:267–282.
- Eales, J. G. 1997. Iodine metabolism and thyroid-related functions in organisms lacking thyroid follicles: Are thyroid hormones also vitamins? *Proc. Soc. Exp. Biol. Med.* 214:302–317.
- Emler, R. B. 1990. World patterns of developmental mode in echinoid echinoderms. Pp. 329–335 in M. Hoshi and O. Yamashita, eds. *Advances in marine invertebrate reproduction*. Vol. 5. Elsevier, Amsterdam.
- . 1995. Developmental mode and species geographic range in regular sea urchins (Echinodermata: Echinoidea). *Evolution* 49:476–489.
- Emler, R. B., and O. Hoegh-Guldberg. 1997. Effects of egg size on postlarval performance: experimental evidence from a sea urchin. *Evolution* 51:141–152.
- Emler, R. B., L. R. McEdward, and R. R. Strathmann. 1987. Echinoderm larval ecology viewed from the egg. *Echinoderm Stud.* 2:55–136.
- Fenaux, L., M. F. Strathmann, and R. R. Strathmann. 1994. 5 Tests of food-limited growth of larvae in coastal waters by comparisons of rates of development and form of echinoplutei. *Limnol. Oceanogr.* 39:84–98.
- Fenaux, L., C. Cellario, and M. Etienne. 1985. Variations in the ingestion rate of algal cells with morphological development of larvae of *Paracentrotus lividus* (Echinodermata, Echinoidea). *Mar. Ecol. Prog. Ser.* 24:161–165.
- Frieden, E. 1981. The dual role of thyroid hormones in vertebrate development and calorigenesis. Pp. 545–563 in L. I. Gilbert and E. Frieden, eds. *Metamorphosis, a problem in developmental biology*. Plenum Press, New York.

- Galton, V. A. 1992. Thyroid hormone receptors and iodothyronine deiodinases in the developing Mexican axolotl, *Ambystoma mexicanum*. *Gen. Comp. Endocrinol.* 85:62–70.
- George, S. B. 1999. Egg quality, larval growth and phenotypic plasticity in a forcipulate seastar. *J. Exp. Mar. Biol. Ecol.* 237: 203–224.
- Gosselin, L. A., and P. Y. Qian. 1997. Can bacterivory alone sustain larval development in the polychaete *Hydroides elegans* and the barnacle *Balanus amphitrite*? *Mar. Ecol. Prog. Ser.* 161:93–101.
- Hadfield, M. G. 2000. Why and how marine invertebrate larvae metamorphose so fast. *Sem. Cell Dev. Biol.* 11:437–443.
- Hanken, J., D. H. Jennings, and L. Olsson. 1997. Mechanistic basis of life history evolution in anuran amphibians: direct development. *Am. Zool.* 37:160–171.
- Hart, M. W., and R. E. Scheibling. 1988. Comparing shapes of echinoplutei using principal components analysis, with an application to larvae of *Strongylocentrotus droebachiensis*. Pp. 277–286 in R. Burke, P. V. Mladenov, P. Lambert and P. L. Parsley, eds. *Echinoderm biology*. A. A. Balkema, Rotterdam.
- Hart, M. W., and R. R. Strathmann. 1994. Functional consequences of phenotypic plasticity in echinoid larvae. *Biol. Bull.* 186: 291–299.
- Havenhand, J. N. 1995. Evolutionary ecology of larval types. Pp. 79–122 in L. R. McEdward, ed. *Ecology of marine invertebrate larvae*. CRC Press, New York.
- Hodin, J., and L. M. Riddiford. 2000. Parallel alterations in the timing of ovarian ecdysone receptor and ultraspiracle expression characterize the independent evolution of larval reproduction in two species of gall midges (Diptera: Cecidomyiidae). *Dev. Genes Evol.* 210:358–372.
- Hodin, J., J. Hoffman, B. J. Miner, and B. J. Davidson. 2001. Thyroxine and the evolution of lecithotrophic development in echinoderms. Pp. 447–452 in M. F. Barker, ed. *Echinoderms 2000*. Balkema, Lisse, The Netherlands.
- Jennings, D. H., and J. Hanken. 1998. Mechanistic basis of life history evolution in anuran amphibians: thyroid gland development in the direct-developing frog, *Eleutherodactylus coqui*. *Gen. Comp. Endocrinol.* 111:225–232.
- Johnson, L. G. 1998. Stage-dependent thyroxine effects on sea urchin development. *N. Z. J. Mar. Freshw. Res.* 32:531–536.
- Johnson, L. G., and C. M. Cartwright. 1996. Thyroxine-accelerated larval development in the crown-of-thorns starfish, *Acanthaster planci*. *Biol. Bull.* 190:299–301.
- Kolding, S., and T. M. Fenchel. 1981. Patterns of reproduction in different populations of five species of the amphipod genus *Gammarus*. *Oikos* 37:167–172.
- Kühn, E. R., and G. F. M. Jacobs. 1989. Metamorphosis. Pp. 187–197 in J. B. Armstrong and G. M. Malacinski, eds. *Developmental biology of the axolotl*. Oxford Univ. Press, New York.
- Lacalli, T. C. 1993. Ciliary bands in echinoderm larvae—evidence for structural homologies and a common plan. *Acta Zool.* 74: 127–133.
- Matsuda, R. 1987. Animal evolution in changing environments with special reference to abnormal metamorphosis. John Wiley and Sons, New York.
- McEdward, L. R. 1985. Effects of temperature on the body form, growth, electron transport system activity, and development rate of an echinopluteus. *J. Exp. Mar. Biol. Ecol.* 93:169–181.
- . 1997. Reproductive strategies of marine benthic invertebrates revisited: facultative feeding by planktotrophic larvae. *Am. Nat.* 150:48–72.
- McEdward, L. R., and D. A. Janies. 1997. Relationships among development, ecology, and morphology in the evolution of echinoderm larvae and life cycles. *Biol. J. Linn. Soc.* 60:381–400.
- McEdward, L. R., and B. G. Miner. 2001. Larval and life cycle patterns in echinoderms. *Can. J. Zool./Rev. Can. Zool.* 79: 1125–1170.
- Morgan, S. G. 1995. Life and death in the plankton: larval mortality and adaptation. Pp. 279–321 in L. R. McEdward, ed. *Ecology of marine invertebrate larvae*. CRC Press, Boca Raton, FL.
- Nijhout, H. F. 1994. *Insect hormones*. Princeton Univ. Press, Princeton, NJ.
- . 1999. Hormonal control in larval development and evolution in insects: Pp. 218–254 in B. K. Hall and M. H. Wake, eds. *The origin and evolution of larval forms*. Academic Press, San Diego, CA.
- Okazaki, K., and K. Dan. 1954. The metamorphosis of partial larvae of *Peronella japonica* Mortensen, a sand dollar. *Biol. Bull.* 106: 83–99.
- Pechenik, J. A. 1987. Environmental influences on larval survival and development. Pp. 551–608 in A. C. Giese, J. S. Pearse, and V. B. Pearse, eds. *Reproduction of marine invertebrates*. Vol. 42. Academic Press, New York.
- Pechenik, J. A., D. E. Wendt, and J. N. Jarrett. 1998. Metamorphosis is not a new beginning. *Bioscience* 48:901–910.
- Perron, F. E. 1986. Life history consequences of differences in developmental mode among gastropods in the genus *Conus*. *Bull. Mar. Sci.* 39:485–497.
- Pfennig, D. W. 1992. Proximate and functional causes of polyphenism in an anuran tadpole. *Funct. Ecol.* 6:167–174.
- Ragan, M. A. 1981. Chemical constituents of seaweeds. Pp. 589–626 in C. S. Lobban and M. J. Wynne, eds. *The biology of seaweeds*. Vol. 17. Blackwell, London.
- Rose, C. S. 1999. Hormonal control in larval development and evolution in amphibians. Pp. 167–217 in B. K. Hall and M. H. Wake, eds. *The origin and evolution of larval forms*. Academic Press, San Diego, CA.
- Roughgarden, J. 1989. The evolution of marine life cycles. Pp. 270–330 in M. W. Feldman, ed. *Mathematical evolutionary theory*. Princeton Univ. Press, Princeton, N.J.
- Rumrill, S. S. 1990. Natural mortality of marine invertebrate larvae. *Ophelia* 32:163–198.
- Saito, M., M. Seki, S. Amemiya, K. Yamasu, T. Suyemitsu, and K. Ishihara. 1998. Induction of metamorphosis in the sand dollar *Peronella japonica* by thyroid hormones. *Dev. Growth Diff.* 40: 307–312.
- Schlichting, C. D. and M. Pigliucci. 1998. Phenotypic evolution: a reaction norm perspective. Sinauer, Sunderland, MA.
- Schlosser, G. 2001. Using heterochrony plots to detect the dissociated coevolution of characters. *J. Exp. Zool.* 291:282–304.
- Strathmann, M. F. 1987. Reproduction and development of marine invertebrates of the northern Pacific Coast. Univ. of Washington Press, Seattle, WA.
- Strathmann, R. R. 1974. Introduction to function and adaptation in echinoderm larvae. *Thalassia Jugoslavica* 10:321–339.
- . 1977. Egg size, larval development, and juvenile size in benthic marine invertebrates. *Am. Nat.* 111:373–376.
- . 1978. Evolution and loss of feeding larval stages of marine invertebrates. *Evolution* 32:894–906.
- . 1985. Feeding and nonfeeding larval development and life history evolution in marine invertebrates. *Ann. Rev. Ecol. Syst.* 16:339–361.
- . 1987. Reproduction and development of marine invertebrates of the northern Pacific Coast. Univ. of Washington Press, Seattle, WA.
- Strathmann, R. R., L. Fenaux, and M. F. Strathmann. 1992. Heterochronic developmental plasticity in larval sea urchins and its implications for the evolution of nonfeeding larvae. *Evolution* 46:972–986.
- Suyemitsu, T., M. Saito, and K. Ishihara. 1997. Thyroid hormones and metamorphosis of sea urchins. Pp. 381–386 in S. Kawashima and S. Kikuyama, eds. *Advances in comparative endocrinology. Proceedings of the 13th international congress of comparative endocrinology*. Monduzzi editore s.p.a, Bologna, Italy.
- Tsuchiya, Y. 1969. Comparative hypocholesterolemic activities of marine algae: Pp. 747–757 in R. Margalef, ed. *Proceedings of the 6th international seaweed symposium*. Subsecretaria de la Marina Mercante, Madrid, Spain.
- Underwood, A. J. 1997. Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge Univ. Press, Cambridge, U.K.
- Vance, R. R. 1973a. Reproductive strategies in marine benthic invertebrates. *Am. Nat.* 107:339–352.
- . 1973b. More on reproductive strategies in marine benthic invertebrates. *Am. Nat.* 107:353–361.
- Wray, G. A. 1995. Evolution of larvae and developmental modes.

Pp. 413–448 in L. R. McEdward, ed. Ecology of marine invertebrate larvae. CRC Press, Boca Raton, FL.

———. 1996. Parallel evolution of nonfeeding larvae in echinoids. *Syst. Biol.* 45:308–322.

Yaoita, Y., and D. D. Brown. 1990. A correlation of thyroid hormone receptor gene expression with amphibian metamorphosis. *Genes Dev.* 4:1917–1924.

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APPENDIX

ANOVA with simple contrast of morphological character measurements (see Fig. 1) as a function of rudiment stage for all experimental treatments compared to CONTROL (morphometrics experiment, approx. 13°C). The comparison is based on observed means. Values represent the mean difference between morphological character measurements (negative values: smaller than CONTROL; positive values: larger than CONTROL).

APPENDIX

ANOVA with simple contrast of morphological character measurements (see Fig. 1) as a function of rudiment stage for all experimental treatments compared to CONTROL (morphometrics experiment, approx. 13°C). The comparison is based on observed means. Values represent the mean difference between morphological character measurements (negative values: smaller than CONTROL; positive values: larger than CONTROL).

Values \pm 1 SE		Rudiment stage			
Treatment	Character	1	2	3	4
HIGH TH (10^{-9} M thyroxine)	Postoral arm	-26 \pm 17	-110 \pm 18**	-146 \pm 39**	-214 \pm 24**
	Postdorsal arm	-27 \pm 24	-63 \pm 24**	-76 \pm 49	-211 \pm 26**
	Body midline	2 \pm 10	-49 \pm 19**	-74 \pm 21**	-162 \pm 21**
	Stomach size	-13 \pm 11	-44 \pm 20*	-104 \pm 23**	-107 \pm 21**
LOW TH (10^{-11} M thyroxine)	Rudiment size	24 \pm 10**	-11 \pm 23	-50 \pm 28	-18 \pm 23
	Postoral arm	-25 \pm 13	-8 \pm 18	9 \pm 37	2 \pm 26
	Postdorsal arm	-20 \pm 19	52 \pm 24*	22 \pm 47	54 \pm 29
	Body midline	-16 \pm 8*	-22 \pm 19	-16 \pm 20	-13 \pm 23
LOW INHIBITOR (10^{-4} M thiourea)	Stomach size	-16 \pm 9	-23 \pm 19	-29 \pm 22	-30 \pm 22
	Rudiment size	-2 \pm 8	-14 \pm 22	-22 \pm 26	37 \pm 25
	Postoral arm	-53 \pm 14**	-57 \pm 18**	-75 \pm 41	-53 \pm 24*
	Postdorsal arm	-50 \pm 20**	2E-02 \pm 24	-51 \pm 51	-72 \pm 26**
HIGH INHIBITOR (10^{-2} M thiourea)	Body midline	-27 \pm 9**	1 \pm 19	13 \pm 21	10 \pm 21
	Stomach size	-17 \pm 9	19 \pm 20	8 \pm 24	53 \pm 20**
	Rudiment size	-21 \pm 8**	-3 \pm 23	-2 \pm 29	16 \pm 23
	Postoral arm	-54 \pm 13**	-93 \pm 15**	-73 \pm 41	N/A
	Postdorsal arm	-144 \pm 18**	-112 \pm 20**	-71 \pm 51	N/A
	Body midline	-12 \pm 8	31 \pm 16	38 \pm 21	N/A
	Stomach size	6 \pm 9	64 \pm 16**	55 \pm 24*	N/A
	Rudiment size	-11 \pm 8	17 \pm 19	-1 \pm 29	N/A

* $P < 0.05$; ** $P < 0.025$.