

Ultimate cause(s) of dwarfism in invertebrates: the case of driftwood talitrids

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ABSTRACT

Question: What are the ultimate cause(s) of dwarfism in talitrids (Crustacea, Amphipoda, Talitridae)?

Hypotheses: Dwarfism evolved: (1) to allow talitrids to negotiate small burrows in driftwood made by the primary colonizers of driftwood; (2) because driftwood was a poor quality food; or (3) to minimize oxygen uptake from seawater in small burrows.

Experimental organisms: *Platorchestia platensis* – driftwood acclimated; *Platorchestia platensis* – wrack acclimated; and *Orchestia gammarellus* – wrack acclimated.

Methods: (1) Behavioural, using maze experiments to test the ability of talitrids of a wide range of body sizes to exit from a circular hole of known diameter. (2, 3) Measurements of oxygen uptake rate from seawater by driftwood- and wrack-acclimated *P. platensis*.

Results: (1) Maze escapes by talitrids were size sorted, such that adults of *O. gammarellus* were too large, whereas adults of the smaller *P. platensis* could complete most of their life cycle within small burrows made by primary colonizers of driftwood. (2) Oxygen uptake rate was significantly lower in driftwood-acclimated than in wrack-acclimated *P. platensis*, consistent with driftwood being a poor quality food. (3) Model predictions of the dissolved oxygen in seawater in burrows showed that both small and large talitrids would be asphyxiated in all available driftwood burrow sizes if they were closed systems.

Conclusions: Results support both hypotheses (1) and (2), but not (3), as possible causes of dwarfism in talitrids.

Keywords: behavioural experiments, invertebrate dwarfism, *Orchestia gammarellus*, oxygen uptake, *Platorchestia platensis*.

INTRODUCTION

Dwarfism is found among both vertebrate and invertebrate animals. For the latter, common examples include dwarf males, as in some spiders (Danielson-François *et al.*, 2012), beetles (Jordal *et al.*, 2002), and barnacles (Sawada *et al.*, 2015). Dwarfism also occurs where both sexes are dwarfs and here the mechanism does not require sexual selection or natural selection of

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sexual systems to be postulated as the evolutionary mechanism of dwarfism. Crustacean dwarfism is probably caused by natural selection. It has been reported among pinnotherid crabs (Decapoda, Pinnotheridae) living commensally in the mantles of some bivalves, or burrows of ghost shrimps (McDermott, 2006); among gammaridean amphipods, including one stenothoid, one pardaliscid, and two species of calliopids (De Broyer, 1977); and among the talitrids (Amphipoda, Talitridae) that live in burrows within rotting marine driftwood (Wildish, 1982).

Seven species of driftwood-specialist talitrids have been described in three genera (Wildish, 2014) among ~250 other talitrid species (Serejo and Lowry, 2008). The most common talitrid ecological groupings are associated with marine, supralittoral wrack and in leaf litter in rainforests (Wildish, 1988). (Wrack consists of macroalgae cast ashore.) Previous studies have established that at least two of the currently known genera of driftwood-specialist talitrids have evolved by dwarfism, namely *Macarorchestia* and *Neotenorchestia* (Wildish, 1982, 2014; Pavese *et al.*, 2014). The third taxon, *Platorchestia chathamensis* (Bousfield, 1982), is known from a single female specimen and little is known about its biology. Wildish (1972) showed that dwarfism was achieved by a slowing of growth in the driftwood-specialist talitrid *Macarorchestia roffensis*, compared with the wrack-generalist species that is most closely related. Reduced growth resulted from:

- extending each inter-moult period;
- reducing the number of moults in each life history;
- sexualization beginning at an earlier moult number;
- size increment at each moult remaining unchanged.

These are the key driftwood-specialist adaptations of talitrid dwarfism and they appear to be present in all known species of *Macarorchestia*, as well as in the recently described genus *Neotenorchestia*. Wildish (1988) referred to this as neotenous dwarfism; however, because neoteny has become such an overused term, lacking a precise meaning, we abandon its use here.

Wildish and Robinson (2016) report that gribbles (Isopoda, Limnoriidae) initiate the macrofaunal colonization of fresh driftwood in the sea. Gribbles are small (1–10 mm in body length), marine, direct-developing isopods. Menzies (1957) lists more than 50 species worldwide. The current WoRMS list (accessed 2015) contains 63 species in four genera.

Gribbles make round, circular holes that are a few millimetres in diameter and a few centimetres deep. They may be accompanied by the wood boring amphipod, *Chelura* sp. Both gribbles (Kern *et al.*, 2013) and chelurids (Green Extabe, 2013) are able to digest lignocellulose without the aid of symbiotic microorganisms. Only a few animals can do this with their own enzymes. Most primary detritivores of dead wood (both terrestrial and marine) utilize symbiotic microbiota to decompose wood.

Talitrids, which are unable to burrow into fresh driftwood themselves, use burrows made by primary detritivores for shelter and food. Shelter protects them from bird predation, from heating by the sun, and from wave washout.

To investigate the evolutionary cause(s) of dwarfism in driftwood-specialist talitrids, we considered three hypotheses:

1. Dwarfism evolved to enable talitrids to negotiate existing burrows in driftwood made by primary wood borers (Wildish and Robinson, 2016).

2. Because driftwood is a poor quality food (Wildish, 1972), it reduces talitrid growth and leads to dwarfism.
3. The small burrows in driftwood limit the oxygen supply in them enough for dwarfism to evolve as a way to minimize talitrids' oxygen requirements.

MATERIALS AND METHODS

Animals and culture

Talitrids were cultured in large snap-on lid boxes for various times before their use in experiments. We added seawater frequently to ensure that cotton wool, driftwood or wrack was moist and that the relative humidity within each culture unit was high. The culture units were kept in natural day/night lighting and in a heated laboratory maintained at 20–22°C.

Driftwood-acclimated *Platorchestia platensis* (Krøyer, 1845), present in a secondary ecotope, were obtained from Hartley Cove in Passamaquoddy Bay (45°05'15.35"N, 66°55'3.49"W) and cultured at 20–22°C over winter as reported in Wildish and Robinson (2016).

Wrack-acclimated *P. platensis* were collected from a primary wrack ecotope at Indian Point, Passamaquoddy Bay (45°04'27.15"N, 67°02'16.03"W) or Haggerty's Beach, Bay of Fundy (45°07'26.56"N, 66°31'26.08"W). At Haggerty's Beach, we also collected a second wrack-generalist species, *Orchestia gammarellus* (Pallas, 1766), which co-occurred with *P. platensis*. Fresh wrack from Indian Point was used to replace rotting wrack in the culture at least weekly.

Simulated driftwood in air – the maze escape experiments

We set up a series of maze escape experiments with live talitrids to determine which ones could escape through holes of various sizes. Primary wood borers in Passamaquoddy Bay make circular burrows in driftwood with a range of sizes, from 0.6 to 5.0 mm (Wildish and Robinson, 2016). We used a similar range of burrow diameters in our tests.

Each maze consisted of a plastic box with snap-on lid (40 cm long × 23 cm wide × 15 cm high; Sterilite Corp., Townsend, MA, USA). We drilled 20 holes of the same size (2, 3, 4 or 5 mm) at one end of the box and covered the other end in seawater-soaked cotton wool. We used individuals of both *P. platensis* and *O. gammarellus*, collecting them alive from Haggerty's Beach on the day of an experiment.

To begin an experiment, we selected 10–150 talitrids (species unknown) to include as wide a range of body sizes as possible. We placed them in a small bucket, which we suspended in a small volume of filtered seawater that we subsequently swirled around. Then we emptied the bucket's contents into an empty maze box and snapped on its lid. After allowing the seawater to drain from the maze and removing any stray pieces of wrack, we placed it in a larger plastic box with snap-on lid (56 × 37 × 30 cm), supported on 2-cm high wooden strips so that the talitrids could exit the maze through the drilled holes, if able to do so. The larger plastic box served as a humid vivarium lined with seawater-dampened cotton wool and freshly collected wrack as food (Fig. 1A). Talitrids that escaped the box with holes had access to food. Those remaining in the smaller box did not.

A few talitrids died due to damage sustained during capture (we excluded them from counts). In general, however, talitrids survived in both boxes, even though those in the maze boxes had no food for at least 48 hours.

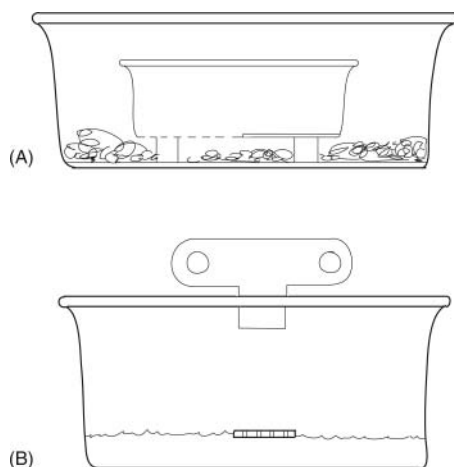


Fig. 1. Diagrams of experimental set-ups for: (A) maze experiment in air with *P. platensis* and *O. gammarellus*; (B) behavioural observations of *P. platensis* in seawater with floating driftwood.

We ended each experiment after 48 hours by collecting all live talitrids in the vivarium (OUT) and those left in maze boxes (IN). We preserved all animals in 50% isopropanol before examining them with a binocular microscope to determine the life-history stage and species of each individual. Sex ratio was determined as in Wildish (1977). To identify the species, we relied on the exopod of uropod 1: *O. gammarellus* has interamal spines on this exopod whereas *P. platensis* does not. We measured photographic images of each individual for total body length (TBL) and body depth (BD) at pereopod 4. Using the software Image Pro Plus v. 5.1, we made accurate calibrated measurements electronically from photographic images (Pavesi *et al.*, 2014) of TBL from the most anterior part of the cephalon to the tip of telson; and BD from the most dorsal part of pereopod 4 to the most ventral point on the coxa. During the 18 days during which the 11 runs of maze escape experiments were performed, continuous temperature recordings in the laboratory (HoboTemp device, Onset Corp., Bourne, MA, USA) averaged 21°C (range 19.8–24.4°C).

Simulated driftwood in seawater

Experiments took place in an aquarium consisting of a Sterilite box with snap-on lid (56 × 37 × 30 cm) to which we added local seawater (psu = 30–32‰) to a depth of 5 cm. The laboratory temperature ranged from 20°C to 24°C during the experiments.

We collected natural, rotted driftwood from Hartley Cove, cut it into lengths of 17–20 cm (10 cm wide by 2–3 cm thick) and drilled it with five 5-mm diameter burrows. We floated one piece of driftwood at a time in the well-illuminated aquarium to simulate driftwood at sea. A time-lapse camera attached to the lid focused on the driftwood. The camera captured images at 2-second intervals (Fig. 1B).

We began each experimental run by turning on the camera and adding 10–30 individuals of *P. platensis*, collected in the morning from Indian Point, to the aquarium. After the experiment was completed, the video was played back and individual frames assessed at one-minute intervals. In each frame, we counted the total number of talitrids on the driftwood and the number on, or in, each of the five artificial burrows.

Oxygen uptake in seawater

Using a size range of driftwood-acclimated *P. platensis*, we measured oxygen uptake of individual talitrids. We used *P. platensis* that were available in April, before the new season's young were born. We collected them from driftwood at Hartley Cove in September 2014 and cultured them over winter with driftwood as their only food.

In addition to the driftwood-acclimated animals, we used wrack-acclimated *P. platensis* from two other locations (Haggerty's Beach and Indian Point), collected in April 2015, to compare the oxygen uptake of driftwood-acclimated talitrids with wrack-acclimated talitrids. In one run of the experiments, we attempted to collect *O. gammarellus* from Haggerty's Beach, where it co-occurs with *P. platensis*.

We transferred the talitrids individually from wrack or driftwood culture to seawater and from there to experimental bottles of 16.56 mL capacity with small pieces of sterile gauze (Spicer and Taylor, 1987) also added to the bottle. We kept the bottles in a water bath in a dark incubator at 21°C and allowed animals to acclimate for 2 hours. We measured the oxygen consumption of a single talitrid in the experimental bottles filled to the brim with locally available, autoclaved seawater (psu = 30‰). We began by sealing the bottles with plastic stoppers to exclude air, then noting the temperature and oxygen content of the seawater. We also ran controls (i.e. bottles with gauze but no talitrid).

As the talitrid clung to the gauze, we measured resting oxygen depletion with a fibre optic oxygen probe with Optrode sensors attached to the bottle walls and recorded by Fibox 3 software on a laptop computer. We made three to five measurements of oxygen concentration during a period of 3–5 hours and we did not allow the oxygen concentration to fall below 50%. We then killed each animal, preserved it in 50% isopropanol, and determined its life-history stage and species, its total body length and body depth, as well as its wet and dry weights (on a micro-balance).

Statistical analysis

We used SPSS™ v. 16.0.1 (IBM Corp., Armonk, NY, USA). To compare means of total body length or body depth between amphipod species or habitat acclimation types, we used a univariate analysis of variance, after ensuring equality of error variance between groups by Levene's test. Non-parametric statistics were not required as the assumptions were all met. To standardize for the effect of size (either total body length or dry weight), we used analysis of covariance provided by the general linear model function. The maximum probability (alpha) for the statistical significance of comparisons was taken to be 0.05, but lower values were reported when present.

RESULTS

Simulated driftwood in air – the maze escape experiments

The maze experiments took place over 18 days in June 2015. Larger burrow diameters led to a greater percentage of wrack-generalists escaping from the maze (Table 1). Only a few of small size were able to negotiate the 2-mm diameter burrow, with the cut-off body depth being 1.9 mm, whereas most of all sizes could escape from the 5-mm diameter burrow. Our results are consistent with the idea that body depth is the limiting factor determining

the talitrid body sizes that can negotiate a simulated gribble burrow. The regression lines of Fig. 2 are:

$$P. platensis: y = 0.1147x + 0.5486, R^2 = 0.76, N = 207$$

$$O. gammarellus: y = 0.1117x + 0.9072, R^2 = 0.55, N = 61$$

There was a significant difference between the two species (GLM, $F = 20.673$, d.f. = 1, $P < 0.001$).

Because of simple reflex behaviours, such as thigmotactic and scototactic responses (Scapini, 2006; Cohen and Putts, 2013) and photonegativity during the day, talitrids such as *P. platensis* and *O. gammarellus* can occupy dark and humid spaces such as simulated gribble burrows. However, the body shape and size of talitrids can limit occupation of the burrows. Body depth at the fourth pereaeon gives a good indication of the size of burrow that a talitrid can negotiate. Talitrid wrack generalists, such as *O. gammarellus*, in the largest adult

Table 1. Maze test results with wrack-adapted *P. platensis* and *O. gammarellus* of different total body lengths (TBL) and body depths (BD)

Variable	Test hole diameter (mm)			
	2	3	4	5
Talitrid TBL range tested (mm)	6.2–23.0	5.8–26.8	5.6–28.1	6.7–26.7
Talitrid BD range tested (mm)	1.0–3.1	1.2–4.7	1.0–4.3	1.3–4.0
Total <i>N</i> tested	99	126	308	28
Maze escape (%)	3	77	90	96
Maze escape, maximum TBL (mm)	10.5	20.5	26.3	27.7
Maze escape, maximum BD (mm)	1.9	2.9	4.2	4.0

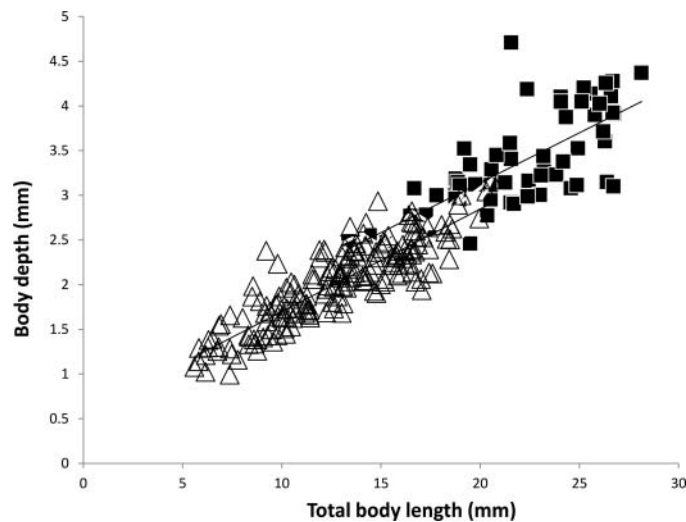


Fig. 2. Scattergram for wrack-acclimated talitrids from Haggerty's Beach: body depth (mm) at pereopod 4, and total body length (mm). $\triangle$, *P. platensis*; $\blacksquare$, *O. gammarellus*.

stages reach maxima of: TBL = 28.133 mm and BD = 4.37 mm in males, and TBL = 26.025 mm and BD = 4.125 mm in females (Table 2). Sexually mature individuals within *O. gammarellus* first occur at TBL > 16 mm (BD equivalent of 2.710 mm), so most of their reproductive life history cannot be completed within gribble burrows. The smaller wrack-generalist *P. platensis* has a better chance of doing this because its mature stage begins at a TBL of ≈ 12 mm (BD equivalent of 1.9293 mm) up to a maximum of 20.588 mm, equivalent to $BD_{\max} = 3.177$ mm in females (data in Fig. 2). So simply because of their smaller size, *P. platensis* are more suited for life in driftwood within gribble burrows.

Simulated driftwood in seawater

Platorchestia platensis showed thigmotactic responses, swimming close to the plastic walls. They were also attracted to the driftwood, crawled onto it and were seen to crawl in and out of the simulated gribble burrows. Results of analysing a subset of the time-lapse images are shown in Table 3. Exploring the holes was a relatively common behaviour linked to photo-negativity and to the generalized escape response from visual predators of talitrids during the day. Of the talitrids that crawled onto the driftwood, 3.6–8.9% were found in or near one of the simulated burrows. In a few cases, the talitrids remained stationary in the burrow entrance for up to 10 minutes.

Table 2. Size comparison (mm) of two wrack-generalist species from Haggerty's Beach, as used in the maze experiments and collected between 2 and 17 June 2015

Statistic	<i>O. gammarellus</i>		<i>P. platensis</i>	
	TBL	BD	TBL	BD
<i>N</i>	61	61	207	207
Mean	22.007	3.325	12.693	1.996
SD	3.586	0.523	3.530	0.457
Median	22.374	3.186	12.824	2.034
Maximum	28.133	4.370	20.588	3.177
Minimum	13.372	2.459	5.605	0.991

Note: TBL = total body length, BD = body depth.

Table 3. Behavioural observations of *P. platensis* on floating driftwood and at entrance/exit of simulated gribble burrows

Run #	Time (minutes analysed)	Number images analysed	Talitrids on driftwood per image			Talitrid burrow entrances/exits per image		
			Mean	Range	Total <i>N</i>	Mean	Range	Total <i>N</i>
1	49	50	8.36	4–16	418	0.74	0–3	37
2	189	190	6.24	0–11	1186	0.27	0–1	51
3	188	189	19.66	4–29	3716	0.74	0–4	140

Driftwood as a low-quality food

Oxygen uptake rates of *P. platensis* were lower for driftwood-acclimated than wrack-acclimated treatments (Figs. 3 and 4). This is consistent with a finding that driftwood is a poorer quality food source and therefore results in slower metabolism, and lower growth and smaller size (Table 4).

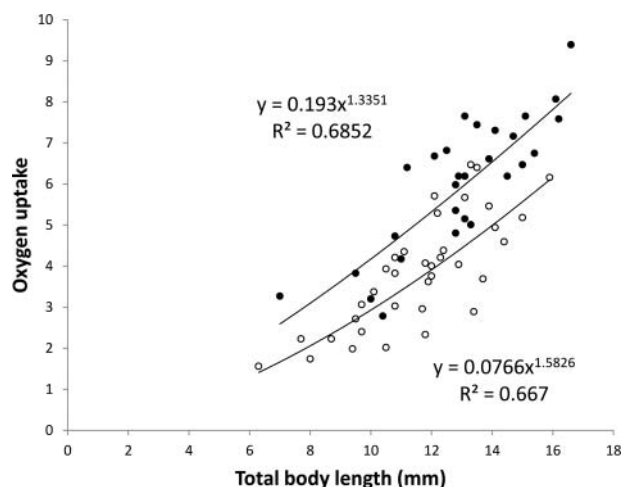


Fig. 3. The relationship between total body length (mm) and oxygen uptake rate (μL O₂ per individual per hour) at 21°C and psu = 30‰ ●, wrack-acclimated *P. platensis*; ○, driftwood-acclimated *P. platensis*.

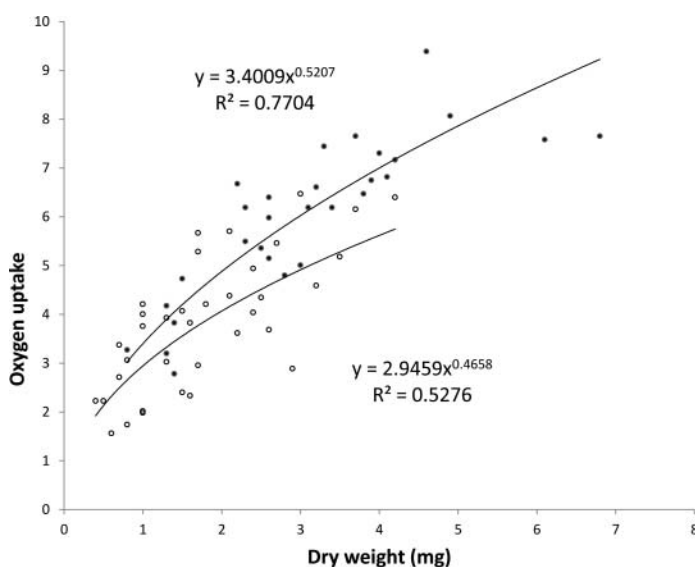


Fig. 4. Oxygen uptake rate (μL O₂ per mg dry weight per hour) as a function of dry body weight (mg) at 21°C and psu = 30‰ ●, wrack-acclimated *P. platensis*; ○, driftwood-acclimated *P. platensis*.

Table 4. Size comparison (mm) of *P. platensis* from Hartley Cove (driftwood-acclimated) and Indian Point or Haggerty's Beach (wrack-acclimated), as used in the oxygen uptake experiments and collected between 4 and 29 April 2015

Statistic	Wrack-acclimated		Driftwood-acclimated	
	TBL	BD	TBL	BD
<i>N</i>	32	32	42	42
Mean	12.692	1.819	11.496	1.723
SD	2.338	0.396	2.092	0.268
Median	12.993	1.868	11.828	1.728
Maximum	16.551	2.652	15.946	2.162
Minimum	6.292	0.985	6.258	1.135

Note: TBL = total body length, BD = body depth.

Oxygen uptake in seawater

Smaller talitrids used less oxygen during resting metabolism (Figs. 3 and 4). Our results agree with the physiological power law – that is, the greater surface area to mass of small animals results in greater oxygen uptake rates per unit mass. Covariance analysis shows that the slope values in both Fig. 3 (GLM, $F = 34.362$, d.f. = 1, $P < 0.001$) and Fig. 4 (GLM, $F = 9.079$, d.f. = 1, $P = 0.004$) are significantly different between wrack- and driftwood-acclimated *P. platensis*.

Comparing *P. platensis* used in the oxygen uptake experiment (Table 4) showed that body size (TBL) was significantly smaller in the driftwood-acclimated group (GLM, $F = 5.36$, d.f. = 1, $P < 0.05$). This is in line with their lower oxygen uptake rates. By contrast, the difference between wrack- and driftwood-acclimated BD in *P. platensis* was not significant (GLM, $F = 1.514$, d.f. = 1, $P = 1.514$).

Given the same environmental conditions, the oxygen uptake rates of wrack-acclimated *P. platensis* and *O. gammarellus* were approximately the same. Our results of 25 April 2015 (Table 5) were compromised because we were able to collect few *O. gammarellus* from Haggerty's Beach. Although the two species were both present, *O. gammarellus* was much less common, and the pebble beach on which they occur made collecting undamaged, live individuals difficult. Nevertheless, Table 5 shows that the two species had broadly overlapping oxygen uptake rates (mg O₂ per hour) and there was no significant difference between their oxygen uptake rates (GLM, $F = 0.012$, d.f. = 1, $P = 0.913$).

Two sets of factors may influence oxygen uptake in talitrids: environmental (including different feeding histories) and genetic (Spicer, 1998). Our experiments were unable to distinguish between them, although it is unlikely that genetic differences are involved in our results since the talitrids collected were from the same local genetic stock.

Table 5. Oxygen uptake rates for wrack-acclimated *P. platensis* and *O. gammarellus* collected on 25 April 2015 from Haggerty's Beach (temperature = 21°C, psu = 30‰)

Life-history stage	TBL (mm)	BD (mm)	Dry weight (mg)	O ₂ · h ⁻¹	O ₂ · mg ⁻¹ · h ⁻¹
<i>P. platensis</i>					
F	14.087	1.825	4.0	7.3025	1.8256
M	16.066	2.144	4.9	8.0676	1.6464
F	14.505	1.981	3.4	6.1898	1.8205
J	7.009	1.008	0.8	3.2688	4.0860
IM	12.491	2.209	4.1	6.8157	1.6624
F	14.957	2.652	3.8	6.4680	1.7021
F	14.735	2.208	4.2	7.1634	1.7056
IF	9.981	1.418	1.3	3.1992	2.4609
F	15.136	2.483	6.8	7.6503	1.1250
F	13.273	1.705	3.0	5.0075	1.6692
F	16.227	2.564	6.1	7.5807	1.2427
F	13.902	1.936	3.2	6.6071	2.0647
IF	11.583	1.623	2.3	5.4943	2.3888
<i>O. gammarellus</i>					
F	20.83	3.034	9.2	9.1340	1.0130
IM	16.076	1.857	3.5	8.0676	2.3050
IF	11.102	1.760	1.7	3.9642	2.3319
IM	13.257	1.955	2.8	9.6672	3.4526
F	16.618	2.513	4.8	6.8852	1.4344
J	7.191	1.135	0.9	1.8778	2.0864

Notes: J = juvenile, IF = immature female, IM = immature male, F = mature female, M = mature male.

DISCUSSION

Physiology of digestion in talitrids

Studies on the physiology of digestion in wrack-generalist talitrids have demonstrated that cellulases are present, but their origin, either from the animal or symbiotic gut microorganisms, has not been conclusively established (Agrawal, 1961; Wildish and Poole, 1970; Johnston *et al.*, 2005). Koop *et al.* (1982) believe that talitrids on a sandy beach are bactivorous, cropping the wrack for the surface-attached microflora. Similar studies in driftwood-specialist talitrids have not been undertaken, so we do not know whether there are significant digestive physiological differences between wrack-generalist and driftwood ecotypes, although the indications are that the digestive physiology of the two is similar. Wildish and Robinson (2016) show that the wrack-generalist *P. platensis* can be cultured for more than 7 months solely on driftwood, suggesting that it already possesses the enzymes – either from endo-symbiotic microbial or from native talitrid sources – required to digest rotting wood. In *O. gammarellus*, Moore and Francis (1985) showed that natural feeding included angiosperm plant material in addition to macroalgal wrack. This is consistent with the finding that the digestive capability in wrack generalists and driftwood specialists is similar and depends, at least in part, on symbiotic microorganisms, but that it is inducible according to the wide range of plant food eaten by talitrids (Johnston *et al.*, 2005).

Modelling oxygen availability in gribble burrows

To minimize dissolved oxygen uptake during occupation of gribble burrows in driftwood at sea, talitrids might become small, thereby minimizing overall oxygen uptake. A typical gribble burrow is 20 mm long (Menzies, 1957), hence the volume for a 3-mm diameter burrow is 141.3716 mm^3 , or 0.1413 mL. The volume of a mature adult *P. platensis* of TBL = 15 mm is 50 mm^3 (Wildish and Frost, 1991), or 0.05 mL. Subtracting this from the gribble burrow volume leaves 0.0913 mL of seawater for oxygen supply. Extrapolating the volume from smaller talitrids (Wildish and Frost, 1991) to larger ones with a TBL = 22 mm, yields a value of 0.0838 mL. Is there enough oxygen to sustain talitrids that are either 15 mm or 22 mm long in the small volume of seawater in the burrow? The total oxygen available in the 3-mm gribble burrow is $5.6 \mu\text{L O}_2 \times \text{available burrow volume} = 0.62 \mu\text{L O}_2$ (given that $5.6 \mu\text{L O}_2 \cdot \text{mL}^{-1}$ is the equilibrium seawater oxygen content at the temperature and pressure measured). If one assumes that the system is closed and that complete oxygen depletion will cause death, then from Fig. 3 we judge that an adult driftwood-acclimated talitrid of 15 mm TBL (resting oxygen uptake = $0.0988 \mu\text{L O}_2 \cdot \text{min}^{-1}$) will consume all the oxygen in the burrow in 5.2 minutes. Similar estimates for different gribble burrow diameters and two sizes (TBL) of driftwood-acclimated talitrids are shown in Table 6. The larger one of 22 mm TBL is the predicted value from the regression equation, although this size does not occur for *P. platensis* in nature – if it did, it would deplete oxygen within a closed, 3-mm diameter burrow in 1.1 minutes.

The burrow is not a closed system, however; it is open to diffusion from air or aerated seawater. Furthermore, in any liquid, talitrids begin beating their pleopods – a paratonic response for all seawater-immersed, amphibious talitrids. The pleopods of *O. gammarellus* beat at 168 strokes per minute over a wide range of oxygen tensions; this rate does not fall until a critical point when air saturation dips below 25% (Walshe-Maetz, 1952). Both diffusion and pleopod beating would enhance oxygen exchange within the burrow and prevent depletion within it. An actively beating pleopod is thus the adaptive response of amphibious marine/estuarine talitrids during the seawater phase of their dispersal, rather than dwarfism to reduce overall oxygen uptake rate.

Table 6. Estimates of time taken by *P. platensis* of two sizes to deplete oxygen in gribble holes of different diameter, assuming that the burrow is a closed system

Burrow diameter (mm)	TBL = 15 mm			TBL = 22 mm		
	Burrow volume less talitrid volume, mL (a)	Total O ₂ in gribble burrow 5.6 (a), $\mu\text{L O}_2$ (b)	Time (min) for O ₂ depletion (b)/0.0988, $\mu\text{L O}_2 \cdot \text{min}^{-1}$	Burrow volume less talitrid volume, mL (a)	Total O ₂ in gribble burrow 5.6 (a), $\mu\text{L O}_2$ (b)	Time (min) for O ₂ depletion (b)/0.0988, $\mu\text{L O}_2 \cdot \text{min}^{-1}$
2	0.0128	0.0717	0.7	—	—	—
3	0.0914	0.5118	5.2	0.0575	0.3220	1.1
4	0.2013	1.1273	11.4	0.1674	0.9374	3.4
5	0.3427	1.9191	19.4	0.3087	1.7287	6.3
6	0.5155	2.8868	29.2	0.4814	2.6958	9.8

Note: TBL = total body length.

Serial evolution of dwarfism in talitrids in which driftwood is the primary ecotope

Five species of the driftwood-specialist genus *Macarorchestia* are known from the northeast Atlantic: *microphthalma* (10.7 mm), *remyi* (8.2 mm), *pavesiae* (7.5 mm), *roffensis* (6.6 mm), and *martini* (5.9 mm), where the numbers in parentheses indicate mean total body length of females. Molecular genetic data shows that *Macarorchestia* is a monophyletic genus, that *microphthalma* is the oldest species, and that the species evolved in order of their TBL, growing smaller and smaller with time (Wildish *et al.*, 2012). Such serial dwarfism prompts us to look for adaptive pressures that might have caused species to become successively smaller.

Gribble burrow diameters in Passamaquoddy Bay (Wildish and Robinson, 2016) – and presumably at other locations – provide an environmental gradient of considerable interest. Gribble burrows ranged from 0.5 to 5 mm with a peak at 2 mm, and an inverse power function describing the frequency of diameter (Wildish and Robinson, 2016). On the other hand, if the adaptive pressure is the low quality of driftwood as talitrid diet, there is no similar environmental gradient. Here the adaptive pressure is a constant and digital in nature, providing no hypothetical explanation of the progressive evolution of smaller body sizes in *Macarorchestia*. Thus the circumstantial, experimental evidence presented here suggests that dwarfism in specialist driftwood talitrids could be an adaptation to the size of available burrows previously constructed by primary colonizers of driftwood, such as gribbles. Dwarfism also enhances many secondary beneficial effects in driftwood specialists such as *Macarorchestia* (i.e. to better utilize a lower quality food and to utilize less oxygen when driftwood is submerged in seawater).

CONCLUSIONS

Of the three mechanisms tested experimentally as a possible cause of dwarfism in talitrids, we were able to eliminate one, hypothesis #3: that the small burrows in driftwood so limit the oxygen supply in them that talitrid dwarfism evolved to minimize their oxygen requirements. This hypothesis fails because both small and larger driftwood talitrids are similarly compromised by oxygen availability in gribble burrows, and all sizes have rapidly beating pleopods to irrigate and aerate the burrows and small spaces they occupy when submerged in seawater (as occurs during dispersal).

This leaves two environmental factors to consider: a range of small burrow diameters in driftwood, and driftwood as a poor diet. They are independent biotic factors and potentially both could affect talitrid evolution and for which dwarfism appears to be an answer.

We cannot list and discriminate all possible environmental factors that may have led to the evolution of serial dwarfism. It is problematic to determine among all factors (both those considered and not considered here), which is the ultimate environmental cause of dwarfism. For talitrids, it is only the smaller sizes of *Macarorchestia* (TBL < 15 mm) and *Platorchestia* (TBL < 20 mm) that can pass all, or most, of their life-history stages within driftwood. In contrast, the larger *Orchestia* species, such as *O. gammarellus*, are inhibited from completing their life history within gribble burrows because as sexually mature adults they are too large for most of the available burrows.

The ecological considerations of importance to talitrids living inside driftwood are three-fold: they are released from predation by shorebirds, they are provided with a long-distance dispersal mechanism, in the form of floating driftwood, and a long-lasting, although low-quality, food source. Because they lack the necessary enzymes, talitrids are unable to

penetrate fresh driftwood unless burrows, made by primary driftwood burrowers, are also present. Two classes of driftwood talitrids have been recognized herein: those where wrack is the primary ecotope, and driftwood is a rare secondary ecotope, such as in *P. platensis*, and those obligately associated with wet rotting driftwood as a primary ecotope such as *Macarorchestia* and *Neotenorchestia* (Wildish, 2014).

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