



Contents lists available at ScienceDirect

## Deep-Sea Research I

journal homepage: [www.elsevier.com/locate/dsri](http://www.elsevier.com/locate/dsri)

## Activity syndromes and metabolism in giant deep-sea isopods

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## ARTICLE INFO

## Keywords:

Bathynomus giganteus  
 Booralana tricarinata  
 Behavioural syndromes  
 Deep-sea isopods  
 Personality

## ABSTRACT

Despite growing interest, the behavioural ecology of deep-sea organisms is largely unknown. Much of this scarcity in knowledge can be attributed to deepwater animals being secretive or comparatively 'rare', as well as technical difficulties associated with accessing such remote habitats. Here we tested whether two species of giant marine isopod (*Bathynomus giganteus*, *Booralana tricarinata*) captured from 653 to 875 m in the Caribbean Sea near Eleuthera, The Bahamas, exhibited an activity behavioural syndrome across two environmental contexts (presence/absence of food stimulus) and further whether this syndrome carried over consistently between sexes. We also measured routine metabolic rate and oxygen consumption in response to a food stimulus in *B. giganteus* to assess whether these variables are related to individual differences in personality. We found that both species show an activity syndrome across environmental contexts, but the underlying mechanistic basis of this syndrome, particularly in *B. giganteus*, is unclear. Contrary to our initial predictions, neither *B. giganteus* nor *B. tricarinata* showed any differences between mean expression of behavioural traits between sexes. Both sexes of *B. tricarinata* showed strong evidence of an activity syndrome underlying movement and foraging ecology, whereas only male *B. giganteus* showed evidence of an activity syndrome. Generally, individuals that were more active and bolder, in a standard open arena test were also more active when a food stimulus was present. Interestingly, individual differences in metabolism were not related to individual differences in behaviour based on present data. Our study provides the first measurements of behavioural syndromes and metabolism in giant deep-sea isopods.

## 1. Introduction

Over the past 20 years, studies have shown that personality (Réale et al., 2007) or behavioural syndromes (Sih et al., 2004) are common, if not ubiquitous, across a wide range of vertebrate and invertebrate taxa (Gosling, 2001; Kralj-Fiser and Schuett, 2014). This broad scope is understandable given that personality traits such as activity, boldness and sociability are representative of fundamental axes of every animal's behavioural repertoire (e.g. antipredator behaviour, habitat use, mating, foraging; Réale et al., 2007). Yet despite this wealth of information, entire genera and indeed ecosystems remain unstudied from a behavioural ecology standpoint. For example, deep sea environments and the organisms therein are particularly neglected in the current literature regarding individual-based differences in behaviour. This scarcity is due partly to animals in these environments being secretive, cryptic,

or comparatively 'rare' (Ramirez-Llodra et al., 2010; Rex and Etter, 2010) but primarily, this can be attributed to technical difficulties associated with accessing, observing, and sampling such remote environments (Clauss and Hoog, 2002; Duarte, 2012; Jamieson et al., 2006). Generally speaking, the majority of deep-water research is directed towards ascertaining species abundance and biodiversity (Barord et al., 2014; Brooks et al., 2011; Grassle, 1991; McLean et al., 2015; Zintzen et al., 2012), with comparatively little attention given to obtaining behavioural measurements *in situ* (there are exceptions, particularly in fishes e.g. Jamieson et al., 2006), or even in the laboratory, as many, particularly vertebrate, species rarely survive transportation to the surface due to thermal, barometric, and physical trauma associated with generalized capture methods (e.g. trawling, long-lining). To combat such challenges, technological innovations such as Baited Remote Underwater Cameras (BRUVs), often incorpo-

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rated into landers (see Bagley et al. (2004) and Priede and Bagley (2000)), have been employed to measure species assemblages and abundances in a non-invasive and low impact manner (Henriques et al., 2002; McLean et al., 2015). However, while this approach is advantageous with respect to population ecology, and can provide repeated measurements at broad scales (Cappo et al., 2006), the applicability of such technologies to studies aimed at individual-based research is rather limited, leaving live targeted collection and testing under controlled laboratory conditions the only reliable method for obtaining repeated individual measurements of wild deep-sea animals.

Here we tested whether or not two species of giant marine isopod (*Bathynomus giganteus*, *Booralana tricarinata*) exhibited consistent individual level differences in activity across two environmental contexts (presence/absence of food stimulus) using the syndrome framework (Sih et al., 2004), and further whether this behaviour carried over consistently between sexes. We also measured routine metabolic rate (RMR) and oxygen consumption ( $\text{MO}_2$ ) in response to a food stimulus in *B. giganteus* to assess whether these variables are related to individual differences in personality. Marine isopods are amenable to this research as they are extremely diverse (Poore and Bruce, 2012) and can be readily caught using a variety of methods, including trapping (Barradas-Ortiz et al. (2003) and references therein). Notably, these taxa are extremely robust, experiencing low mortality following targeted capture (but see Talwar et al. (2016) for higher estimates as fisheries bycatch) and acclimate readily to laboratory conditions making them rare, but ideal candidates for behavioural study under controlled conditions. Our choices in terms of study species were based on the facts that both *B. giganteus* and *B. tricarinata* are found throughout the Exuma Sound, with sampling sites being situated near (approx. 1 km offshore) our research facilities on Eleuthera, The Bahamas. We predicted that both species would exhibit consistency along the activity axis of personality (an activity syndrome) but that within each species there would be sex-specific variation in overall level of activity. This prediction is based on the notion that trade-offs likely exist between males and females with differing reproductive strategies, physiological needs, and potentially, life-expectancy or survivorship (Réale et al., 2010). Quantifying the existence of across-context carryover in activity measurements in these two species of marine isopod, as well as aspects of metabolism in *B. giganteus*, represent the first attempts to measure individual behavioural consistency and its proximate basis in a species from a deep-sea environment. As such, our study provides rare ecological and evolutionary insights into an environmental realm heretofore largely unstudied in behavioural ecology.

## 2. Methods

### 2.1. Field collection

Both *Bathynomus giganteus* (N = 12, Mass (g): 181–1097 [mean = 367.3], Total Length (TL): 17.6–35.8 cm; primary collection site 1) and *Booralana tricarinata* (N = 18, Mass (g): 2.4–8.0 [mean = 4.6], TL: 4.7–6.4; primary collection site 2), were collected from two sites (Site 1 – LAT: 24.783611, LONG: -76.34324480613833 [trap depth 653 m, temperature 11.6 °C]; Site 2 – LAT: 24.776852, LONG: -76.34067970219488 [trap depth 875 m, temperature 6.2 °C]) using deep-sea traps in the northeast Exuma Sound offshore of the island of Eleuthera (Fig. 1), The Bahamas in January, 2015. These size distributions fall within normal range for these species in the area (*B. giganteus*, TL: 8.9–34.5 cm; *B. tricarinata*, TL: 2.9–8.3 cm; M. Violich unpublished data) and region in general (*B. giganteus*, TL: 7–34 cm; Magalhães and Young, 2003). The trapping rigs included a square trap, a cylindrical trap, and a temperature depth recorder (Lotek LAT-1400, Newfoundland, Canada) in a series on a main line (Fig. 2a). The square trap (61×61×46 cm) consisted of a 2.5 cm<sup>2</sup>

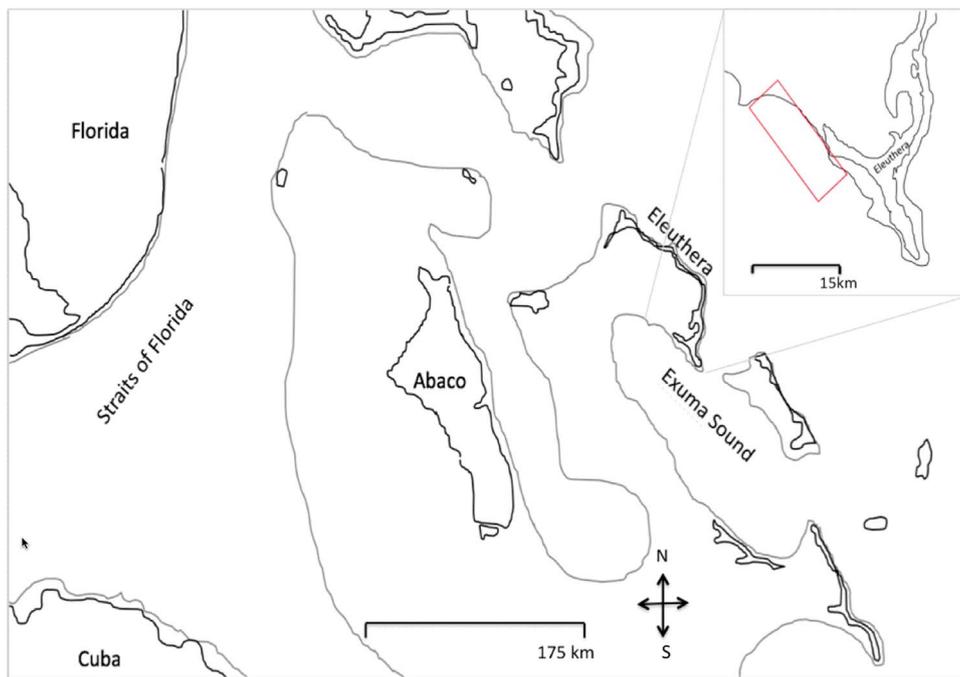
plastic-covered wire mesh enclosure with two entrance funnels (26 cm deep and 15 cm diameter) and two square doors (25×25 cm) (Fig. 2b). The cylindrical trap (height: 26 cm, diameter: 40 cm) consisted of a 1.3 cm<sup>2</sup> plastic-covered wire mesh enclosure with one entrance funnel (37 cm deep, opening diameter 6 cm) and two square doors (16×14 cm) (Fig. 2c). Traps were baited with frozen little tunny (*Euthynnus alletteratus*) and deployed for approximately 24 h prior to retrieval using an onboard electric pot hauler (duration 45–90 min depending on trap resting depth).

Following field collection, isopods were immediately placed in chilled coolers containing seawater and brought back to the research facilities at the Cape Eleuthera Institute. Upon arrival, individuals were sexed (the presence of functional oostegites in females and appendices masculinae in males), measured for body size (mass), and marked using unique combinations of 2 visible elastomer implant tags (Northwest Marine Technology Inc.) inserted into the basis region of one pereopod (Fig. 3). All individuals had eaten at time of capture (evidenced though stomach distention/contents being visible through translucent exoskeleton). Isopods were allowed to acclimate to our (species-specific) communal holding conditions in one of three identical insulated, white, aerated, rectangular chilling containers (90×35×35 cm; water depth 20 cm; temperature: 11 ± 1 °C) for a minimum of 48 h prior to oxygen consumption testing (*B. giganteus*) or 72 h prior to the onset of behavioural testing (both species). Individuals were rotated among all holding containers throughout the study. As one of our experimental trials involved response to a food stimulus, individuals were not provided food in holding and only during testing (see below).

### 2.2. Respirometry

An intermittent-flow respirometry system (Loligo Systems, Hobro, Denmark) was used to measure the oxygen consumption ( $\text{MO}_2$ ) and routine metabolic rate (RMR) of individual *B. giganteus*. Due to differences in body size/mass two separate chamber sizes and corresponding cycle durations were used for individuals separated into two groups (n=7, mean 419 g, range of 197–1097 g; and n=5, mean 243.6, range of 181–318 g respectively) including an 8-chamber system (14 L volume; 13.6 °C ± 0.03) and a 4-chamber system (0.95 L volume; 13.2 °C ± 0.05). To maintain a consistent cold temperature, chambers received aerated sea water that was recirculated through a stainless steel coil in an ice bath before being reintroduced into the experimental setup. Due to equipment limitations in our field laboratory we were not able to lower our respirometry chamber temperature below 13 °C, however this temperature is similar to the at-depth temperature of the primary capture site for *B. giganteus* (site 1, 11.6 °C) and all individuals were thermally acclimated in their respective chamber for a minimum of 24 h on a constant flush cycle prior to the onset of data collection. All transfers of individuals occurred in darkness with minimal ambient red-light to minimize stress related to handling and transfer from holding to the respirometry apparatus.

Oxygen probes were calibrated using oxygen depleted seawater (sodium sulfite) and fully aerated seawater before each trial. For the 8 chamber system containing larger individuals, the intermittent flow through system was set to a cycle with a 10 min flush period, a 30 s wait period, and a 30 min recirculation cycle for the measurement period. Due to their smaller mass and corresponding chamber size, the 4 chamber system was set to a cycle with a 10 min flush period, a 60 s wait period, and a 15 min recirculation cycle for the measurement period. Oxygen content was recorded in each chamber for every second of the measurement period using an oxygen probe (Loligo Systems). After the initial > 24 h acclimatization period,  $\text{MO}_2$  was monitored for at least 5 h. Each chamber was then injected with 3 ml of a feeding chemical cue (blood from *E. alletteratus*) at the start of a measurement period. Injections were conducted in complete darkness via in-water conduits to minimize physical disturbance and injections to all

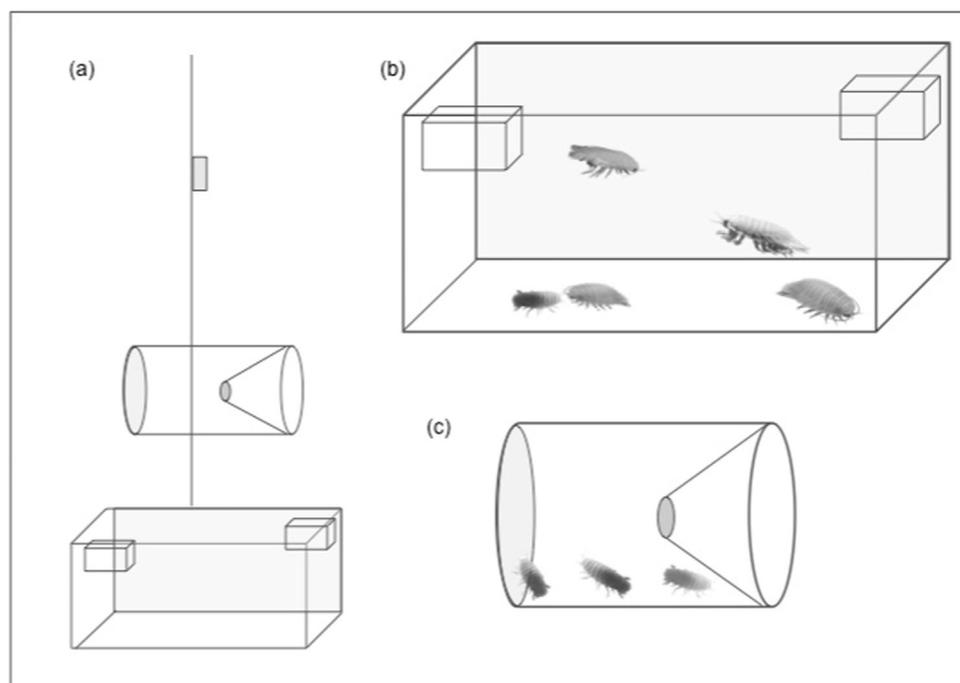


**Fig. 1.** Map of Exuma Sound, southwest of the island of Eleuthera, The Bahamas. Photo inset illustrates isopod collection area ~1 km offshore. The grey lines represent the border of the continental shelf.

chambers was complete in less than 1 min total. Recirculation times varied from 25 to 30 min for individuals in the larger 8 chamber system to 10–15 min for individuals in the smaller 4 chamber system. The next flush period flushed the water completely out of the system to ensure there was no residual chemical cue in subsequent measuring periods. After the flush of the chemical cue,  $MO_2$  continued to be recorded for 11 h in total darkness. After the trials, animals were removed from their chambers and returned to their respective holding containers. Background, microbial respiration was measured in empty chambers and accounted for in the  $MO_2$  calculations.

### 2.3. Behavioural testing

Prior to the onset of behavioural testing, individual isopods were selected haphazardly from the holding containers and placed into one of six rectangular experimental arenas. Two of the experimental arenas were identical to the large communal holding tanks described above, and used solely for testing the larger *B. giganteus* while the remaining four arenas were scaled down in size (55×30×30 cm, water depth 10 cm) but identical in terms of construction and conditions (temperature:  $10 \pm 1$  °C) for testing of *B. tricarinata*.



**Fig. 2.** Collection apparatus (a) used for field trapping of *B. giganteus* and *B. tricarinata*, including (b) a rectangular trap and (c) a circular trap attached to a main rigging line.



**Fig. 3.** Photograph depicting isopod tagging procedure using two visible elastomer implant tags (Northwest Marine Technology Inc.) inserted into the basis region of one pereopod, to allow for individual identification.

Behavioural trials involved two sets of experimental treatments for each individual, each night, over the course of three days (3 replicates). All trials occurred between 18:00 and 22:00 and involved two 30 min activity trials in the presence/absence of food stimulus. Treatment 1 (T1) involved placing an individual in the experimental arena and quantifying the total amount of time spent active (s) and mean duration of activity (s), as a measure of intermittent locomotion (Wilson and Godin, 2010) for each individual. To avoid confounding measurements associated with an escape response resulting from initial handling, the first 60 s of each 30 min trial was not included in data analyses. Treatment 2 (T2) was identical to the first, with the only modification being the addition of a small piece of cut fish (~30 g, *E. alletteratus*) to the opposite end of the arena immediately prior to the focal individual being added to the trial. As above, total time spent active (s) and mean duration of activity (s) were quantified, however total time spent feeding (manipulating the food item) was also recorded.

Due to water cooling limitations at the field laboratory, it was not possible to entirely replace seawater in the experimental tanks between trials, though partial changes were made. As such, our experimental order was fixed in that we performed all T1 trials prior to conducting the second set of T2 trials to avoid confounding our results between treatments. In addition, substrate was not provided in any of the experimental or holding arenas to prevent chemical cues being retained between the first and second sets of trials, as well as to facilitate later video analysis. In all instances, every individual used in the experiment had prior experience (> 24 h) in the experimental arenas prior to data collection and as such we were measuring activity, not exploratory behaviour per se (Réale et al., 2007). All trials were recorded using GoPro 2 action cameras (Struthers et al., 2015), with weak ambient

lighting being provided by red LED lights (< 5 lm). Behavioural attributes were quantified continuously over the 30 min trial using the behavioural analysis software JWatcher (v1.0).

#### 2.4. Data and statistical analyses

First, we compared mean activity metrics and mass measurements between sexes and species using the nonparametric Mann-Whitney *U* test. As we did not observe any significant differences between sexes in any behavioural metrics (see results) in either species, our second set of comparisons focused on within and between species comparisons across traits using the Spearman's rank correlation test. Lastly, to test our initial predictions regarding the presence of an activity syndrome between sexes and species, all behavioural metrics were collapsed into first principal component scores for each treatment/context using Principal Components Analysis (PCA) separately for each species (Table 1). We then obtained across-context correlations between the PC1 score for T1 and the score for T2 using Spearman's rank correlation test as above. Mean values were used to generate a more accurate measurement and account for habituation over repeated behavioural trials.

Unfortunately, the oxygen probes failed for several individuals during the measurement period before the injection cue was introduced. As such, routine metabolic rate (RMR) was determined as the average  $MO_2$  for 11 h overnight after the chemical cue injection. Peak  $MO_2$  was the highest  $MO_2$  value after the chemical cue injection.

### 3. Results

#### 3.1. Sex- and species-based differences in mass and behavioural metrics

In general, we did not observe any significant differences between sexes in the mean time spent active (T1 & T2), mean duration of activity bouts (T1 & T2), mean foraging duration (T2), and body size (mass) in either *B. giganteus* (range  $Z_1 = -1.19$ – $0.39$ , all  $P > 0.05$ ), nor *B. tricarinata* (range  $Z_1 = -1.01$ – $1.26$ , all  $P > 0.05$ ) (Table 2). This trend also carried over for most behavioural traits between species (range  $Z_1 = -1.13$ – $1.08$ , all  $P > 0.05$ ) with the exception of time spent active in the presence of a food stimulus ( $Z_1 = -2.85$ ,  $P = 0.0039$ ) where *B. tricarinata* were more than twice as active as *B. giganteus*. As expected, *B. giganteus* were significantly larger than *B. tricarinata* ( $Z_1 = 4.57$ ,  $P < 0.0001$ ).

#### 3.2. Correlations and syndrome analysis

##### 3.2.1. *Bathynomus giganteus*

In general, most behavioural attributes were not significantly associated with one another.

or with body size (mass) ( $r_s = -0.39$ – $0.61$ ,  $P > 0.05$ ). However, individuals that were more active in the baseline trial (T1) were also

**Table 1**

PCA loadings of within-context behavioural metrics used to generate first principal component scores (PC1) to assess across-context correlations in activity in *B. giganteus* and *B. tricarinata*.

Behavioural Context	Metrics within each context (mean)	Loadings for PC1	Percentage of variation explained
Activity (T1) – <i>B. giganteus</i>	Time spent active (s)	0.9764	95.3
	Duration of activity bout T1 (s)	0.9764	
Activity in presence of food stimulus (T2) – <i>B. giganteus</i>	Time spent active (s)	0.9804	68.9
	Feeding duration (s)	0.8990	
	Duration of activity bout T1 (s)	0.5459	
Activity (T1) – <i>B. tricarinata</i>	Time spent active (s)	0.9510	90.4
	Duration of activity bout T1 (s)	0.9510	
Activity in presence of food stimulus (T2) – <i>B. tricarinata</i>	Time spent active (s)	0.9117	59.4
	Feeding duration (s)	0.5209	
	Duration of activity bout T1 (s)	0.8248	

**Table 2**Mean ( $\pm$  SD) measurements for mass, feeding duration, and activity metrics across treatments in male and female *B. giganteus* and *B. tricarinata*.

	Species (sex)			
	<i>B. tricarinata</i> (male) [N =9]	<i>B. tricarinata</i> (female) [N =8]	<i>B. giganteus</i> (male) [N =7]	<i>B. giganteus</i> (female) [N =5]
Mass (g)	4.6 $\pm$ 1.8	4.6 $\pm$ 1.2	386.8 $\pm$ 342.2	265 $\pm$ 60.8
Activity T1 (s)	196.9 $\pm$ 141.8	153.6 $\pm$ 132.5	146.4 $\pm$ 174.1	88 $\pm$ 31.1
Activity T2 (s)	310.7 $\pm$ 159.8	283.9 $\pm$ 145.6	113.4 $\pm$ 108.3	53.6 $\pm$ 24.2
Feeding duration T2 (s)	23 $\pm$ 24.5	75.8 $\pm$ 98.2	43.8 $\pm$ 73	14.6 $\pm$ 25.4
Duration of activity bout T1 (s)	17.8 $\pm$ 9.2	12.7 $\pm$ 9.4	21.6 $\pm$ 14.7	23 $\pm$ 9.7
Duration of activity bout T2 (s)	31.3 $\pm$ 38.5	21.2 $\pm$ 12.5	31.4 $\pm$ 8.2	24.9 $\pm$ 9.7

**Table 3**

Spearman correlations in activity metrics and between treatments in two species of marine isopod.

Trait 1	Trait 2	<i>B. tricarinata</i>		<i>B. giganteus</i>	
		$r_s$	P value	$r_s$	P value
Activity T1 (s)	Activity T2 (s)	0.7132	0.0013	0.7333	0.0246
Activity T2 (s)	Feeding duration T2 (s)	0.6548	0.0043	–	–
Activity T1 (s)	Duration of activity bout T1 (s)	0.8260	< 0.0001	0.8667	0.0025
Activity T2 (s)	Duration of activity bout T2 (s)	0.7574	0.0004	–	–
Duration of activity bout T1 (s)	Duration of activity bout T2 (s)	0.5809	0.0145	–	–
Mass (g)	Duration of activity bout T2 (s)	–0.5887	0.0129	–	–

All relevant Spearman correlations at  $P < 0.05$  are shown.

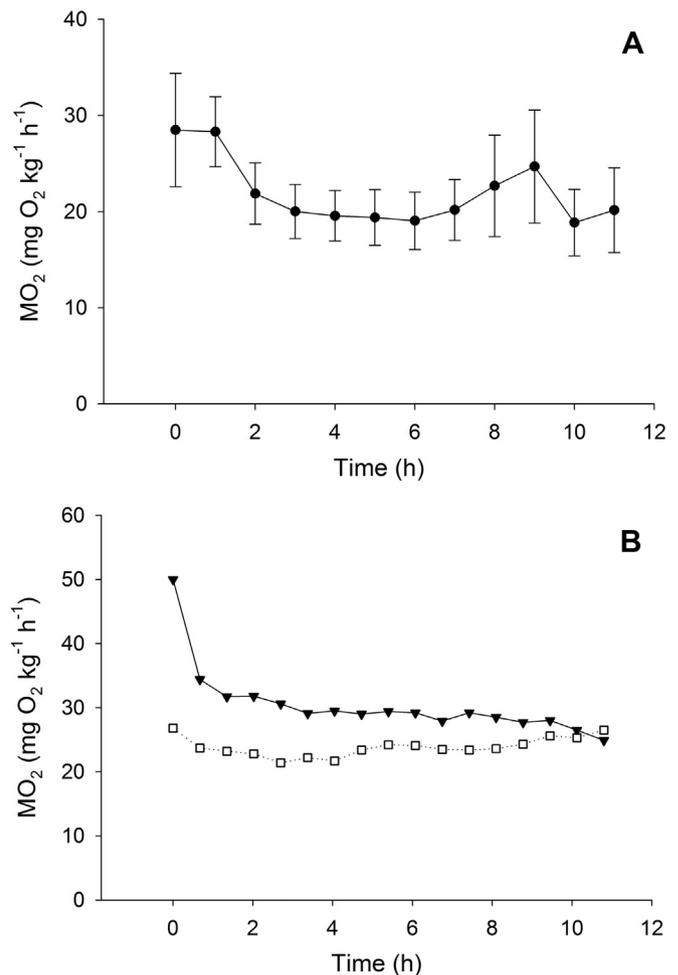
more active in the feeding trials (T2) ( $r_s = 0.7$ ,  $P = 0.024$ ). Similarly, individuals that did tend to be more active in the baseline measurement (T1) also tended to have longer bouts of activity (T1) ( $r_s = 0.9$ ,  $P < 0.001$ ) (Table 3). In addition, our Spearman's rank correlation test between the first PCA scores for both activity treatments did not initially support the prediction of a species-level activity syndrome in *B. giganteus* ( $r_s = 0.548$ ,  $P = 0.16$ ). However, upon examination of the sexes separately, it appears that this initial result might be sex-specific as males do show strong evidence of an activity syndrome ( $r_s = 1.0$ ,  $P < 0.0001$ ), while females do not ( $r_s = -0.5$ ,  $P = 0.667$ ).

### 3.2.2. *Booralana tricarinata*

*Booralana tricarinata* showed strong significant correlations between traits within and between contexts/treatments (Table 3). More active individuals in an empty arena (T1) were more active in the presence of a food stimulus (T2) and individuals that were more active in both treatments also tended to move for longer durations (T1 & T2). Durations of individual bouts of activity were also consistent across treatments and smaller individuals tended to move for longer durations when food was present (T2). Lastly individuals that were more active in T2 also tended to feed for longer durations (Table 3). In contrast to *B. giganteus*, our Spearman's rank correlation tests suggest strong evidence of an activity syndrome for both sexes (males:  $r_s = 0.950$ ,  $P < 0.001$ ; females:  $r_s = 0.857$ ,  $P < 0.001$ ), as well as at the species level ( $r_s = 0.899$ ,  $P < 0.001$ ).

### 3.3. Metabolism and correlations in *B. giganteus*

RMR was  $21.8 \pm 3.4$  mg/kg/h with a range of 8.3 – 34.5 mg/kg/h. Fig. 4a shows the mean hourly  $MO_2$  response to the chemical cue. Peak  $MO_2$  following the chemical cue was  $39.4 \pm 6.1$  mg/kg/h (range: 15.3 – 67.3 mg/kg/h). Interestingly, the  $MO_2$  response to the chemical cue varied across individuals with two distinct response profiles being observed. Some individuals exhibited elevated  $MO_2$  immediately following exposure and a subsequent decline in  $MO_2$  over time, while others displayed no response to the chemical cue (Fig. 4b). A comparison of T2 activity levels between those individuals that responded to the chemical cue, and those that displayed no response (grouped as 'responders' and nonresponders' respectively) did not yield a significant difference in T2 mean activity between groups ( $Z_1 = -0.69$ ,  $P = 0.49$ ).



**Fig. 4.** (A) Mean  $\pm$  SEM hourly oxygen consumption rate ( $MO_2$ ) following presentation of a food stimulus at time 0 for 10 *B. giganteus* and (B) representative traces for 2 individuals, one showing elevated  $MO_2$  in response to the stimulus (black triangles), the other displaying no  $MO_2$  response to the stimulus (white squares).

Neither RMR nor peak  $\text{MO}_2$  were significantly correlated with any behavioural attribute or body size (mass) (RMR:  $r_s = -0.24$ – $0.51$ ,  $P > 0.05$ ;  $\text{MO}_2$ :  $r_s = -0.31$  to  $0.29$ ,  $P > 0.05$ ). As expected, RMR was highly positively correlated with peak  $\text{MO}_2$  ( $r_s = 0.9$ ,  $P = 0.0003$ ) with individuals demonstrating a higher RMR also having a higher peak  $\text{MO}_2$ .

#### 4. Discussion

Our results suggest that two species of giant deep-water marine isopod show an activity syndrome (consistent individual level differences in activity across time/situations) across environmental contexts (presence/absence of food stimulus), but the underlying mechanistic basis of this syndrome, particularly in *B. giganteus*, is unclear. Contrary to our initial predictions, neither *B. giganteus* nor *B. tricarinata* showed any differences between mean expression of behavioural traits between sexes or, for the most part, species. Similarly, both sexes of *B. tricarinata* showed strong evidence of an activity syndrome underlying movement and foraging ecology, whereas only male *B. giganteus* showed this same relationship (when considering sexes separately). Generally, individuals that were more active, and potentially bolder in a standard open arena test (T1), were also more active when a food stimulus was present (T2). Interestingly, individual differences in RMR and  $\text{MO}_2$  were not related to individual differences in behavioural attributes, though experimental limitations regarding sample size and metabolic measurements for all individuals (see below) preclude any strong conclusions from being drawn in this regard. The behavioural dynamics of deep-water marine isopods, like many taxa in this realm, are largely unknown. However, studies of intertidal isopods might offer insight into the results of the present study. Jormalainen and Tuomi (1989) proposed that continuous high levels of activity in male *Idotea balthica*, might offer fitness benefits in terms of locating potential reproductive partners. Similarly, high levels of activity are also advantageous for locating resources for faster growth, and larger body size can be correlated with increased reproductive success in both sexes (Hemmi and Jormalainen, 2004; Jormalainen et al., 1994, 1992). As such, consistent within- and between-individual differences in activity might be related to physiology and life-history traits – key factors necessary for the emergence of ‘personality’ (Careau and Garland, 2012; Careau et al., 2008; Réale et al., 2010). A recent study by Yli-Renko et al. (2015) supports this assertion, demonstrating that *I. balthica* exhibits consistent individual-level variation in activity and further, that these individual-level differences covary with differential over-winter survival.

In our study, we did not observe any differences in behaviour related to body size in *B. giganteus*, but smaller *B. tricarinata* tended to move for longer durations when food was present. Further, we also found that *B. giganteus* and *B. tricarinata* differed in overall activity profiles between treatments. For example, both male and female *B. tricarinata* showed an increase in activity in the presence of a food stimulus (T2) relative to the open arena test (T1), but contrastingly *B. giganteus* showed the opposite pattern, with a notable decline in activity in both sexes (females 39%; males 22%, Table 2) in T2. Further, we also found that both species exhibited consistent patterns of inter-individual differences in activity across environmental contexts, as indicated by the activity syndromes in males of both species and female *B. tricarinata*.

The presence of an activity syndrome in *B. giganteus* and *B. tricarinata*, and particularly the observed overall increase in activity in T2 shown by both sexes in *B. tricarinata*, fits well with our current understanding of the ecology of deep-sea isopods. As facultative scavengers, *B. giganteus* and *B. tricarinata* possess a diverse range of adaptations (specialized mouthparts, chemoreceptors, large/flexible guts, and capacity to store lipid reserves in their hepatopancreas and fat body) that are highly specialized for the detection, acquisition, and ingestion of resources arising from unpredictable (in space and time) ‘food falls’ and carrion (Barradas-Ortiz et al., 2003; Britton and

Morton, 1994). These adaptations help isopods cope with the intense inter- and intra-specific competition surrounding patchy resources by providing a manner by which these organisms can maximize food ingestion quickly to avoid predation or cannibalism by larger conspecifics (Barradas-Ortiz et al., 2003; Smith and Baldwin, 1982). Further, we can assume that consistency in behavioural traits that allow for an increased ability to locate and exploit potential food items in a resource poor environment (such as the deep sea) would also be adaptive and potentially ecologically advantageous. Réale et al. (2010) proposed that such links between metabolism and activity might reflect differences in ‘pace-of-life’. For example, individuals with a high metabolism, high growth rate, early reproduction and short life span would show high-levels of activity and risk-taking (boldness), while individuals with a slow pace-of-life would show the opposite relationships. While our behavioural results for *B. giganteus* and *B. tricarinata*, and previous work on *I. balthica* (Yli-Renko et al., 2015) offer supportive evidence of such a notion, we did not observe a link between activity and metabolism (RMR, peak  $\text{MO}_2$  in response to a food cue) in our assessments of *B. giganteus*. Notably, only 11 individuals had the full suite of behavioural and  $\text{MO}_2$  tests so it is difficult to ascertain whether this result is attributable to sample size limitations or reflects an accurate assessment. That said, our  $\text{MO}_2$  measurements correspond proportionally with other previous estimates of  $\text{MO}_2$  in coldwater isopods (e.g. *Glyptonotus antarcticus*, Robertson et al., 2001). Alternatively, isopod activity in the context of foraging and exploration in *B. giganteus* may be linked to metabolism in ways other than that measured in the context of the present study.

Time constraints did not allow us to measure standard metabolic rate (SMR), which represents the minimum energetic requirements of a resting, postabsorptive, non-reproducing, thermally-acclimated animal to sustain life. In addition, maximum metabolic rate (MMR) was not assessed (e.g. via exercising the animals to exhaustion) nor was aerobic scope (MMR – SMR), which is an indication of the aerobic capacity of an animal for activities beyond maintenance such as locomotion, growth and reproduction. These metabolic parameters (SMR, MMR and/or aerobic scope) may play an important mechanistic role in determining activity behavioural syndromes and warrant further investigation (Biro and Stamps, 2010; Careau et al., 2008). Nevertheless, our results are the first concurrent metabolic/personality measurements that we know of for a deep sea species and therefore offer initial insights on this topic for future studies.

We also observed two distinct metabolic responses to the chemical food cue. Some individuals clearly responded to the cue, with an elevated  $\text{MO}_2$  immediately after exposure. This elevation in  $\text{MO}_2$  could have been an activity response in the chamber (i.e. the animal increased movement in the chamber, perhaps searching for food, thus increasing  $\text{MO}_2$ ). Alternatively, the animal may have remained motionless but responded with an upregulation of metabolic pathways related to increased vigilance and/or digestion in anticipation of a meal. The respirometry trials were not recorded visually, so it is difficult to ascertain the cause of this  $\text{MO}_2$  response profile. However, we would predict that the individuals that metabolically responded to the chemical cue would be associated with a behavioural syndrome related to an increase in activity in the presence of a food stimulus (T2). In contrast, other individuals maintained a constant, continuous  $\text{MO}_2$  throughout the entire measurement period, displaying no metabolic response to the food cue. One might predict that those nonresponsive individuals would be associated with having lower activity when a food stimulus is present in the behavioural trials (T2). However, when allocating individuals into two groups represented by ‘responders’ and ‘nonresponders’, we did not observe a significant difference in T2 mean activity between groups and thus cannot support this assertion based on current data.

Another interesting result in our study, though in contrast to our initial predictions, is that female *B. giganteus*, do not show an activity syndrome as found in males or either sex of *B. tricarinata*. While

comparatively few studies have examined sex differences in the context of personality, what studies do exist tend to suggest that males behave more consistently than females (reviewed in Schuett et al. (2010)). While in most respects, males of both species in our study do appear to behave more consistently than females, female *B. tricarinata* also show the same activity pattern as males. While the basis of this discrepancy is unknown, a potential sample size limitation in our study for female *B. giganteus* could, in whole or in part, explain this result. As such, caution should be employed when considering this result more broadly. More difficult to explain is our finding regarding a decline in activity in *B. giganteus* when exposed to a food stimulus. A potential explanation might relate to differences in individual 'state' (Wolf and Weissing, 2010). Since our capture method involved baited traps and *B. giganteus* is larger and thus able to gain access to resources more easily when in competition with smaller heterospecifics (Smith and Baldwin, 1982), it is possible that this species was simply more satiated and less interested in feeding. However, we did not observe a significant difference in mean feeding duration or proportion of individuals feeding between species in our trials. Similarly, our use of two different sized traps, each targeting either *B. giganteus* (rectangular trap) or *B. tricarinata* (circular trap) differentially, should have mitigated this possibility. That being said, traps in general can represent a form of sampling bias (Biro and Dingemans, 2009) but this is not always the case (Michelangeli et al., 2015) and in this instance, the only viable sampling alternatives (e.g. trawls) that might target a broader subset of the population also offer increased risk of stress and injury/mortality (Talwar et al., 2016). Alternatively, one could speculate that this decline in activity might represent some form of food preference or behavioural/ physiological adaptation related to body size or the frequency/proximity of food-falls in a resource-poor environment (i.e. a reduction in overall activity when a food source is detected nearby). Future study is required to ascertain what significance these preliminary results might have for understanding behavioural consistency in these species.

The deep-sea remains one of the least explored environments in behavioural ecology, largely because it is perceived to be inaccessible or its denizens difficult to study in the setting of a laboratory environment. However many taxa from this realm, particularly invertebrates (Kralj-Fiser and Schuett, 2014), are well suited to field capture and laboratory study. Using a simple experimental design, our study provides the first evidence of an activity syndrome or personality, as well as metabolic measurements in deep-sea isopods. Contrary to our initial predictions, neither *B. giganteus* nor *B. tricarinata* showed any differences between mean expressions of behavioural traits between sexes nor, for the most part, species. Our results suggest that two species of giant marine isopods show an activity syndrome across environmental contexts, but the underlying mechanistic bases (e.g. metabolism) of this syndrome, particularly in *B. giganteus*, is unclear. While our results are just the first step in developing an understanding of the behavioural ecology of deep-sea isopods, experimental studies such as ours, in combination with non-extractive, *in situ* technological approaches (Bailey et al., 2002; Bailey et al., 2005; Jamieson et al., 2006; McLean et al., 2015) might provide a way forward for exploring the behavioural ecology of the deep-sea environment. To further understand how personality contributes to isopod ecology, future work should attempt to ascertain the proximate mechanism linking behaviour to physiology and metabolism (Careau and Garland, 2012; Careau et al., 2008; Réale et al., 2010).

### Compliance with ethical standards

#### Funding

This study was supported by an NSERC Discovery Grant (S.J.C.) (315774), the Canada Research Chairs Program (S.J.C.), and Carleton University.

### Conflict of interest

All authors declare that they have no conflicts of interest.

### Ethical approval

This research was performed in accordance with the laws, guidelines, and ethical standards of the country in which they were performed (the Bahamas) and also adheres to the guidelines of the Canadian Council on Animal Care and the laws of Canada. As such, all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

### Acknowledgements

The authors gratefully acknowledge the Cape Eleuthera Institute for providing facilities and support for this research. We also thank Z. Zuckerman for assistance with respirometry.

### References

- Bagley, P.M., Priede, I.G., Jamieson, A.D., Bailey, D.M., Battle, E.J.V., Henriques, C., Kemp, K.M., 2004. Lander techniques for deep-ocean biological research. *Underw. Technol.* 26, 3–12. <http://dx.doi.org/10.3723/175605404783101567>.
- Bailey, D.M., Jamieson, A.J., Bagley, P.M., Collins, M.A., Priede, I.G., 2002. Measurement of in situ oxygen consumption of deep-sea fish using an autonomous lander vehicle. *Deep Sea Res. Part I: Oceanogr. Res. Pap.* 49, 1519–1529. [http://dx.doi.org/10.1016/S0967-0637\(02\)00036-5](http://dx.doi.org/10.1016/S0967-0637(02)00036-5).
- Bailey, David M., Genard, Bertrand, Collins, Martin A., Rees, Jean-François, Unsworth, Susan K., Battle, Emma J.V., Bagley, Philip M., Jamieson, Alan J., Priede, Imants G., 2005. High swimming and metabolic activity in the deep-sea eel *Synaphobranchus kaupii* revealed by integrated *in situ* and *in vitro* measurements. *Physiol. Biochem. Zool.* 78, 335–346. <http://dx.doi.org/10.1086/430042>.
- Barord, G.J., Dooley, F., Dunstan, A., Ilano, A., Keister, K.N., Neumeister, H., Preuss, T., Schoepfer, S., Ward, P.D., 2014. Comparative Population Assessments of Nautilius sp in the Philippines, Australia, Fiji, and American Samoa Using Baited Remote Underwater Video Systems. *Plos One* 9. <http://dx.doi.org/10.1371/journal.pone.0100799>.
- Barradas-Ortiz, C., Briones-Fourzán, P., Lozano-Álvarez, E., 2003. Seasonal reproduction and feeding ecology of giant isopods *Bathynomus giganteus* from the continental slope of the Yucatán peninsula. *Deep Sea Res. Part I: Oceanogr. Res. Pap.* 50, 495–513. [http://dx.doi.org/10.1016/S0967-0637\(03\)00036-0](http://dx.doi.org/10.1016/S0967-0637(03)00036-0).
- Biro, P.A., Dingemans, N.J., 2009. Sampling bias resulting from animal personality. *Trends Ecol. Evol.* 24, 66–67. <http://dx.doi.org/10.1016/j.tree.2008.11.001>.
- Biro, P.A., Stamps, J.A., 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends Ecol. Evol.* 25, 653–659. <http://dx.doi.org/10.1016/j.tree.2010.08.003>.
- Britton, J.C., Morton, B., 1994. Marine carrion and scavengers. *Oceanogr. Mar. Biol. Annu. Rev.*, 369–434.
- Brooks, E.J., Sloman, K.A., Sims, D.W., Danylchuk, A.J., 2011. Validating the use of baited remote underwater video surveys for assessing the diversity, distribution and abundance of sharks in the Bahamas. *Endanger. Species Res.* 13, 231–243.
- Cappo, M., Harvey, E.S., Shortis, M., 2006. Counting and measuring fish with baited video techniques: an overview. *Aust. Soc. Fish. Biol. Workshop Proc.*, 101–114.
- Careau, V., Garland, T., 2012. Performance, personality, and energetics: correlation, causation, and mechanism. *Physiol. Biochem. Zool.: Ecol. Evolut. Approaches* 85, 543–571. <http://dx.doi.org/10.1086/666970>.
- Careau, V., Thomas, D., Humphries, M.M., Réale, D., 2008. Energy metabolism and animal personality. *Oikos* 117, 641–653. <http://dx.doi.org/10.1111/j.0030-1299.2008.16513.x>.
- Clauss, G., Hoog, S., 2002. Deep-sea challenges of marine technology and oceanographic engineering Science-Technology Synergy for Research in the Marine Environment: Challenges for the XXI Century. Elsevier, Amsterdam, 133–142.
- Duarte, C.M., 2012. The Exploration of Marine Biodiversity. Fundación BBVA, Madrid, Spain.
- Gosling, S.D., 2001. From mice to men: what can we learn about personality from animal research? *Psychol. Bull.* 127, 45–86.
- Grassle, J.F., 1991. Deep-sea benthic biodiversity. *Bioscience* 41, 464–469.
- Hemmi, A., Jormalainen, V., 2004. Geographic covariation of chemical quality of the host alga *Fucus vesiculosus* with fitness of the herbivorous isopod *Idotea baltica*. *Mar. Biol.* 145, 759–768. <http://dx.doi.org/10.1007/s00227-004-1360-4>.
- Henriques, C., Priede, I., Bagley, P., 2002. Baited camera observations of deep-sea demersal fishes of the northeast Atlantic Ocean at 15–28°N off West Africa. *Mar. Biol.* 141, 307–314. <http://dx.doi.org/10.1007/s00227-002-0833-6>.
- Jamieson, A.J., Bailey, D.M., Wagner, H.J., Bagley, P.M., Priede, I.G., 2006. Behavioural responses to structures on the seafloor by the deep-sea fish *Coryphaenoides armatus*: implications for the use of baited landers. *Deep Sea Res. Part I: Oceanogr. Res. Pap.* 53, 1157–1166. <http://dx.doi.org/10.1016/j.dsr.2006.04.002>.
- Jormalainen, V., Tuomi, J., 1989. Sexual differences in habitat selection and activity of

- the colour polymorphic isopod *Idotea baltica*. *Anim. Behav.* 38, 576–585. [http://dx.doi.org/10.1016/s0003-3472\(89\)80002-8](http://dx.doi.org/10.1016/s0003-3472(89)80002-8).
- Jormalainen, V., Tuomi, J., Merilaita, S., 1992. Mate choice for male and female size in aquatic isopod *Idotea balthica*. *Ann. Zool. Fenn.* 29, 161–167.
- Jormalainen, V., Merilaita, S., Tuomi, J., 1994. Male choice and male-male competition in *Idotea baltica* (Crustacea, Isopoda). *Ethology* 96, 46–57.
- Kralj-Fiser, S., Schuett, W., 2014. Studying personality variation in invertebrates: why bother? *Anim. Behav.* 91, 41–52. <http://dx.doi.org/10.1016/j.anbehav.2014.02.016>.
- Magalhães, N., Young, P.S., 2003. *Bathynomus A. Milne Edwards, 1879* (Isopoda, Cirolanidae) from the Brazilian coast, with description of a new species. *Arq. do Mus. Nac., Rio De. Jan.* 61, 221–239.
- McLean, D.L., Green, M., Harvey, E.S., Williams, A., Daley, R., Graham, K.J., 2015. Comparison of baited longlines and baited underwater cameras for assessing the composition of continental slope deepwater fish assemblages off southeast Australia. *Deep-Sea Res. Part I-Oceanogr. Res. Pap.* 98, 10–20. <http://dx.doi.org/10.1016/j.dsr.2014.11.013>.
- Michelangeli, M., Wong, B.B.M., Chapple, D.G., 2015. It's a trap: sampling bias due to animal personality is not always inevitable. *Behav. Ecol.* 27, 62–67. <http://dx.doi.org/10.1093/beheco/arv123>.
- Poore, G.C.B., Bruce, N.L., 2012. Global Diversity of Marine Isopods (Except Asellota and Crustacean Symbionts). *PLoS One* 7, e43529. <http://dx.doi.org/10.1371/journal.pone.0043529>.
- Priede, I.G., Bagley, P.M., 2000. *In situ* studies on deep-sea demersal fishes using autonomous unmanned lander platforms. *Oceanogr. Mar. Biol.: Annu. Rev.* 38, 357–392.
- Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C.R., Levin, L.A., Martinez Arbizu, P., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B.E., Smith, C.R., Tittensor, D.P., Tyler, P.A., Vanreusel, A., Vecchione, M., 2010. Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. *Biogeosciences* 7, 2851–2899. <http://dx.doi.org/10.5194/bg-7-2851-2010>.
- Réale, D., Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J., 2007. Integrating animal temperament within ecology and evolution. *Biol. Rev.* 82, 291–318. <http://dx.doi.org/10.1111/j.1469-185X.2007.00010.x>.
- Réale, D., Garant, D., Humphries, M.M., Bergeron, P., Careau, V., Montiglio, P.-O., 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philos. Trans. R. Soc. B: Biol. Sci.* 365, 4051–4063. <http://dx.doi.org/10.1098/rstb.2010.0208>.
- Rex, M.A., Etter, R.J., 2010. *Deep-sea biodiversity: pattern and scale*. Harvard University Press, Cambridge, USA.
- Robertson, R., El-Haj, A., Clarke, A., Peck, L., Taylor, E., 2001. The effects of temperature on metabolic rate and protein synthesis following a meal in the isopod *Glyptonotus antarcticus* Eight (1852). *Polar Biol.* 24, 677–686. <http://dx.doi.org/10.1007/s003000100268>.
- Schuett, W., Tregenza, T., Dall, S.R.X., 2010. Sexual selection and animal personality. *Biol. Rev.* 85, 217–246. <http://dx.doi.org/10.1111/j.1469-185X.2009.00101.x>.
- Sih, A., Bell Alison, M., Johnson, J.C., Ziemba Robert, E., 2004. Behavioral syndromes: an integrative overview. *Q. Rev. Biol.* 79, 241–277. <http://dx.doi.org/10.1086/422893>.
- Smith, K.L., Baldwin, R.J., 1982. Scavenging deep-sea amphipods: effects of food odor on oxygen-consumption and a proposed metabolic strategy. *Mar. Biol.* 68, 287–298. <http://dx.doi.org/10.1007/bf00409595>.
- Struthers, D.P., Danylchuk, A.J., Wilson, A.D.M., Cooke, S.J., 2015. Action cameras: bringing aquatic and fisheries research into view. *Fisheries* 40, 502–512. <http://dx.doi.org/10.1080/03632415.2015.1082472>.
- Talwar, B., Brooks, E.J., Grubbs, R.D., 2016. An assessment of post-release mortality for a commonly discarded deep-sea isopod (*Bathynomus giganteus*) using reflex impairment. *ICES J. Mar. Sci.* <http://dx.doi.org/10.1093/icesjms/fsw087>.
- Wilson, A.D.M., Godin, J.-G.J., 2010. Boldness and intermittent locomotion in the bluegill sunfish, *Lepomis macrochirus*. *Behav. Ecol.* 21, 57–62. <http://dx.doi.org/10.1093/beheco/arp157>.
- Wolf, M., Weissing, F.J., 2010. An explanatory framework for adaptive personality differences. *Philos. Trans. R. Soc. B: Biol. Sci.* 365, 3959–3968. <http://dx.doi.org/10.1098/rstb.2010.0215>.
- Yli-Renko, M., Vesakoski, O., Pettay, J.E., 2015. Personality-dependent survival in the marine isopod *Idotea balthica*. *Ethology* 121, 135–143. <http://dx.doi.org/10.1111/eth.12323>.
- Zintzen, V., Anderson, M.J., Roberts, C.D., Harvey, E.S., Stewart, A.L., Struthers, C.D., 2012. Diversity and composition of demersal fishes along a depth gradient assessed by baited remote underwater stereo-video. *PLoS One* 7, e48522. <http://dx.doi.org/10.1371/journal.pone.0048522>.