Journal of Crustacean Biology Advance Access published 3 May 2021

The Crustacean Society

Journal of Crustacean Biology

Journal of Crustacean Biology (2021) 41(2), 1–9. doi:10.1093/jcbiol/ruab013

Physiological stress and recovery kinetics in trawl escapees of the Antarctic krill *Euphausia superba* Dana, 1850 (Euphausiacea)

Ludvig A. Krag[1], Bjørn A. Krafft[2], Bent Herrmann[1,3,4] and Peter V. Skov[1]

[1]*Danmarks Tekniske Universitet (Technical University of Denmark), 9850 Hirtshals, Denmark;*
[2]*Havforskningsinstituttet (Institute of Marine Research), 5870 Bergen, Norway;*
[3]*SINTEF Ocean, 9850 Hirtshals, Denmark; and*
[4]*Norges Arktiske Universitet (The Artic University of Norway), 9037 Breivika, Tromsø, Norway*

Correspondance: L.A. Krag: e-mail: lak@aqua.dtu.dk

(Received 5 January 2021; accepted 2 April 2021)

ABSTRACT

When caught in a trawl, some individuals interacting with the fishing gear may escape, but such interactions may lead to physiological trauma that causes direct delayed mortality and/or increased vulnerability to predation. Understanding fishery-induced stress levels and the recovery period of escapees is therefore crucial for predicting total fishing-induced mortality. Hemolymph lactate concentration is commonly used as an index of physiological stress in many crustacean species, and the clearing time of lactate back to normal levels indicates the ability to recover from stress. We measured the hemolymph lactate concentration in three groups of Antarctic krill (*Euphausia superba* Dana, 1850): Group 1, trawl escapees collected during fishing; Group 2, specimens subjected to simulated mesh penetration; and Group 3, an onboard acclimated control group. Individuals that had escaped the trawl during fishing had the highest concentrations of hemolymph lactate (mean > 6 $mmol^{-1}$). Exposure to mesh penetration was in itself not stressful, as hemolymph lactate concentrations did not differ significantly between Group 2 and the control Group (mean 0.8 $mmol^{-1}$ *versus* 0.7 $mmol^{-1}$, respectively). Additional stress factors during the capture and handling process likely added to the elevated lactate levels observed in Group 1. For the trawl escapees, the lactate clearance time during stress recovery was modeled as a function of exponential decay. Hemolymph lactate levels did not differ significantly among the three groups after 200 min, which suggested that Antarctic krill recovered from fishery-induced stress within this time period.

Key Words: escape mortality, krill fishery, lactate, pelagic trawl, stress clearance, stress response

INTRODUCTION

Mortality studies of trawl-net escapees usually measure cumulative mortality rate after several days of holding, but quantitative information about stress levels or rates of recovery are rarely considered. Stress levels, however, may be so severe that they exert a direct lethal effect on the caught animals (Ryer, 2004; Broadhurst *et al.*, 2006), and sub-lethal stress or exhaustion may have indirect lethal effects, such as greater vulnerability to predation. The magnitude of such effects is not accounted for in traditional survival experiments in which escapees are monitored in protective holding facilities where they are isolated from potential predators. Biochemical and physiological analyses of stress responses are needed to better understand causes of mortality associated with fishing and for improving harvesting technology.

Measuring changes in hemolymph chemistry has been the primary means for assessing stress in crustaceans associated with commercial capture and live holding (reviewed in Stoner, 2012a). The stress response in crustaceans involves the rapid release of crustacean hyperglycemic hormone (CHH) from the sinus-x gland (Stoner, 2012a). The physiology and actions of CHH are complex, as this hormone has a multitude of functions related to metabolism, moulting, and osmoregulation. Among these actions is the mobilization of glucose and fatty acids to the hemolymph (Fanjul-Moles, 2006) to satisfy increased energy demands during stress. Quantifying CHH levels in the hemolymph is not trivial

and traditionally involves the use of radioimmunoassays (Chang *et al.*, 1976) or immunohistochemistry (Chang *et al.*, 1998). The increased glucose levels in the hemolymph are ultimately subjected to glycolysis to form pyruvate. The anaerobic formation of ATP produces an equimolar amount of lactate when oxygen delivery to the tissues is limited. The use of lactate as an indicator of stress has been validated in numerous studies in which crustaceans have been exposed to stress, including air exposure, handling, and mechanical stress from trawling (Stoner, 2012a). Lactate can therefore be considered a valid proxy for physiological stress, and hemolymph lactate levels could potentially be used as a tool to predict delayed mortality in escaped or discarded crustaceans.

Monitoring the escape mortality of a given fishery provides an estimate of the total mortality following a monitoring period after escapement from a fishing gear. Such an estimate includes the accumulated mortality of all involved stressors, including any injuries sustained during escape from the fishing gear or subsequently during collection, onboard retrieval, or transfer to monitoring facilities. The potential contribution from increased predation on escapees due to temporary physiological impairment is not included in this mortality estimate, but for some species it is considered to be substantial (Ryer, 2004). The degree of physiological impairment is likely proportional to the degree of exhaustion, thus the level of elevation of hemolymph lactate and the subsequent clearance time before lactate levels again normalise are useful proxies for degree of impairment and duration.

Measurements of blood or hemolymph parameters often require costly laboratory equipment and considerable labor investments. Such requirements set boundaries for the parameters that can be assessed using conventional techniques in field locations (Harter *et al.*, 2014). Recently developed portable and easy-to-use clinical analyzing devices, such as i-STAT© (Abbot Point of Care Inc., Princeton, NJ, USA), are nevertheless gaining popularity in biological research for a range of vertebrate and invertebrate species (Harter *et al.*, 2014; Stoot *et al.*, 2014). Such devices allow for rapid analysis of single or multiple blood or hemolymph parameters. As these devices are developed for humans, data cannot always be used indiscriminately, as it may be invalid or require adjustment (Stoot *et al.*, 2014). Point-of-care devices have been used with success in the analysis of lactate in teleost fishes (Brown *et al.*, 2008; Gallagher *et al.*, 2010; Serra-Llinares *et al.*, 2012) for simultaneous measurements of different blood/body-fluid parameters.

Antarctic krill (*Euphausia superba* Dana, 1850, hereafter krill) are an abundant fishery resource (Krafft *et al.*, 2016), and trawlers use pelagic trawl gear with various designs to capture them. Krag *et al.* (2014) demonstrated that several length classes of krill can escape through the commercial trawl mesh sizes currently in use. Krafft *et al.* (2016) quantified the escape mortality of krill escaping a trawl during towing to be 4.4 ± 4.4% after a 60 h holding period, concluding that krill are robust with high tolerance to the capture and escape process. The effects of fishery-induced stress levels and the recovery period of escapees on total fishing-induced mortality, however, are poorly understood. Hemolymph lactate levels have been shown to closely parallel levels of CHH following stress in the Christmas Island red crab (*Gecarcoidea natalis* Pockock, 1888) (Morris *et al.*, 2010), with the possible existence of a feedback loop between lactate levels and CHH release (Fanjul-Moles, 2006). We therefore propose that hemolymph lactate levels in Antarctic krill are also a robust indicator of stress in this species.

Our objective was to measure stress levels and describe the temporal lactate clearing process of: 1) krill individuals that escaped from a trawl cod-end during fishing and were collected in a cod-end cover net and hauled onboard for examination, 2) onboard acclimatised individuals that escaped through commercial netting under controlled conditions, and 3) a group of onboard acclimatised individuals that served as the control group. The experiment was carried out at sea in Antarctica, and measurements were made using a portable point of care i-STAT analyzer to evaluate the hemolymph lactate levels and their relationship to fishing gear-related stress exposure.

MATERIAL AND METHODS

Study site and collection of specimens

This study was carried out on commercial fishing grounds near the shelf of the South Orkney Islands (60°35′S, 45°30′W). FV *Juvel*, a Norwegian, 99.5 m. krill trawler, was used as the research platform. Trawl tows were conducted on acoustic registrations using a covered cod-end methodology to collect individuals escaping the trawl cod end (Wileman *et al.*, 1996). A pelagic macroplankton trawl was fitted with a 7 mm hoop cover surrounding the 15.4 mm (16 mm nominal) commercial mesh-size cod end (see Krafft *et al.*, 2016) to collect escapees.

The trawl escapee Group 1 was collected using short towing durations aimed at small catches. We used this approach to best resemble the escapement process at depth during commercial trawling, as we suspected that larger catches of escaped krill in the cover net might increase mechanical damage and physiological stress due to denser individual packing, potential lack of oxygen, and prolonged handling time. To collect individuals for the on-board controlled mesh-penetration test Group 2 and the control Group 3, an initial haul was conducted by closing the cover and keeping the inside cod end open. See Krafft *et al.* (2016) for further details on on-board holding conditions. A sample of individuals from the initial haul was transferred to eight 15 l aquariums, which were submerged into the four 1000 l onboard holding tanks (two 15 l aquariums in each 1,000 l tank). This population of individuals were used to establish Groups 2 and 3.

Hydrography data were acquired using a mini CTD (www.star-oddi.com) mounted to the trawl beam that logged data at 10 sec intervals. A trawl eye sensor (type A1, www.marport.com) attached to the headline provided depth and temperature information during fishing operations. The towing of the trawl followed commercial speeds of about 2.0–2.5 knots.

Stress measurements

For Group 1, physiological stress measurements were made on individuals from the cod-end cover as soon as the catch was taken onboard for individuals from six of the eight hauls conducted and presented in Krafft *et al.* (2016) in order to couple physiological stress levels directly with escape mortality after 60 h of monitoring (escape survival column (P_{60}) in Table 1 of Krafft *et al.* (2016)). The remaining two hauls in Krafft *et al.* (2016) did not contain sufficient numbers of individuals to be included in this study.

For the controlled mesh-penetration experiment, acclimatised individuals from Group 3 were transferred to a netting cylinder (16 cm in diameter, 26 cm high) with 15.4 mm mesh size commercial netting covering the cylinder walls and bottom (Fig. 1). The cylinder was inserted into an aquarium and manually moved, and individuals that penetrated the meshes of the cylinder to escape into the aquarium (i.e., Group 2) were collected and transferred to a 15 l aquarium and used for repeated lactate measurements.

Time zero (T_0) was recorded as the time at which the trawl arrived on deck (Group 1), time of the controlled mesh-penetration process (Group 2), or time of removal from the 15 l aquariums (Group 3). All subsequent stress measurements were recorded relative to T_0 in the three different groups (Fig. 1). No dead individuals had to be removed from any of the eight holding aquariums 24 h after the krill arrived on board, at which time the control group was considered to be acclimatised.

To measure lactate level, small batches of 4–8 individuals from each group were transferred gently and rapidly from the holding facilities on deck to the onboard laboratory in a 2 l

Table 1. Operational conditions, catch weights of *Euphausia superba* in cover net and cod-end, and survival probability from Krafft *et al.* (2016); *, no data recorded.

Haul	Maximum depth (m)	Haul duration (min)	Minimum temperature (°C)	Salinity (‰) (mean ± SD)	Cover catch (kg)	Cod-end catch (kg)	P60 (Krafft *et al.*, 2016)
1*	–	–	–	–	–	–	–
2	165	34	−1.2	33.3 ± 0.1	0.5	10	1.00
3	126	42	−1.3	33.0 ± 2.7	6	58	0.98
4	191	30	−1.2	33.3 ± 0.3	7	50	0.94
5	93	36	−1.1	31.3 ± 5.6	0.5	9	0.98
6	22	30	0.0	31.1 ± 0.1	15	84	0.90

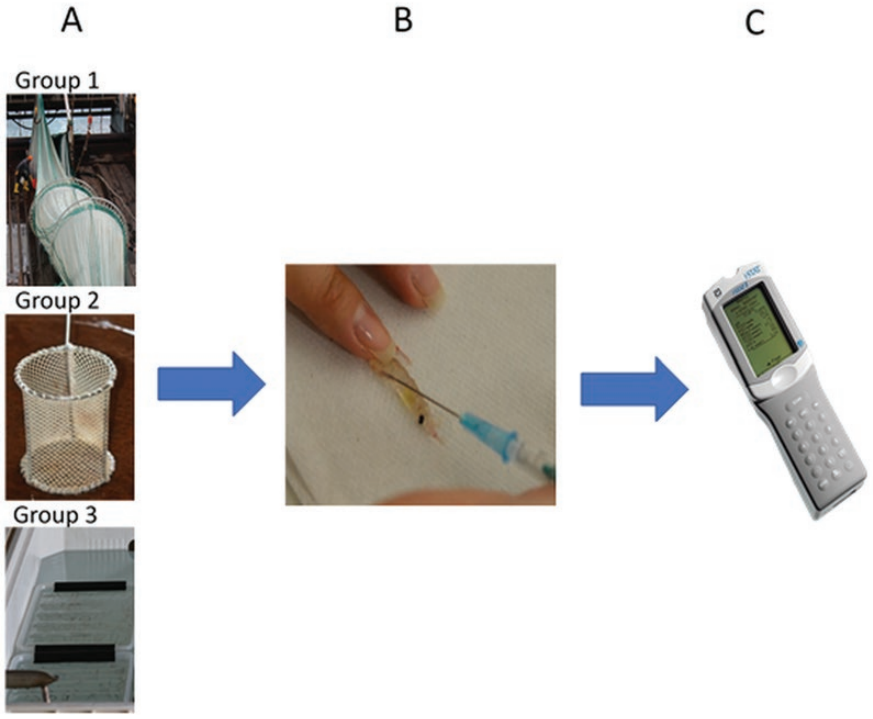

Figure 1. The three experimental groups, trawl escapees (Group 1), controlled onboard escapees (Group 2), and onboard control (Group 3) (**A**). Extraction of hemolymph from *Euphausia superba* using a syringe (**B**) and obtaining lactate measurements from the i-STAT (**C**).

plastic container with seawater. The sampling intensity was high following T_0 where several test measurements were performed within the first hour and decreased thereafter, as the changes in lactate were expected to be highest within the first hours following T_0. Hemolymph was extracted from the krill using a 1 ml Norm Ject Tuberkulin syringe (Buch and Holm, Herlev, Denmark) equipped with a 0.6 mm × 30 mm BD MicrolanceTM3 needle (Buch and Holm) (Fig. 1). When sufficient hemolymph was extracted (95 µl of fluid is required for the device, pooled from 3–5 individuals from the same group), the sample was injected into the CG4+ cartridge, which then was inserted into the i-STAT system for measurement and calculation of lactate values. Exact times for the start and end of each measurement relative to T_0 were recorded.

The i-STAT system was developed primarily for fresh human whole blood at a temperature around 37 °C. The accuracy of blood acid-base status and blood gases are not known, as they are temperature dependent (Harter *et al.*, 2014; Malte *et al.*, 2014). Although the CG4+ cartridge also measures partial pressures of oxygen and CO_2, we only used the lactate values, as to our knowledge the analysis of this parameter is not temperature sensitive.

Statistical analysis

To model the temporal development of lactate concentration $lc(t)$ (in $mmol^{-1}$) for krill in individual hauls, we considered four different models:

$$lc(t) = \begin{cases} c & : Model\ 1 \\ a \times t + c & : Model\ 2 \\ a \times exp(-b \times t) & : Model\ 3 \\ a \times exp(-b \times t) + c & : Model\ 4 \end{cases}$$

where t is the time in minutes after the krill arrived onboard the vessel. Model 1 considers the potential case of no development of lactate concentration as a function of time after arrival, leading to a constant concentration, c. Model 2 considers a potential linear decrease or increase in lactate concentration with time after arrival. Model 3 describes a situation in which the decrease in lactate concentration during the time period after arrival on the vessel is proportional to the concentration at a specific time, which leads to the well-known exponential decay model used to describe

Table 2. Summary of lactate measurements for the three experimental groups of krill (*Euphausia superba*).

Haul	Measurements	Duration	Lactate (mmol–1)	
	(N)	(min.)	Start	End
Trawling (Group 1)				
1	14	1,455	5.49	0.37
2	12	566	6.98	1.26
3	14	3,380	3.16	0.32
4	8	128	6.38	3.94
5	9	179	2.35	1.78
6	11	165	3.59	1.74
Experimental control (Group 2)				
1	6	205	0.52	0.49
2	5	154	0.42	0.63
3	5	167	0.38	0.45
4	5	138	1.45	0.91
5	5	145	0.58	0.73
Experimental mesh penetration (Group 3)				
1	6	169	0.79	0.59
2	5	150	0.61	0.70
3	5	145	0.87	0.65
4	5	132	0.53	0.78
5	6	188	0.40	0.81

many natural processes. Model 3, however, has an asymptotic concentration at zero but the natural concentration of lactate would not be zero. Model 4 considers the asymptotic concentration level as modelled by a constant c while it maintains the exponential decay pattern of Model 3.

All four models were tested for each haul separately, and the model with the lowest Akaike Information Criterion (AIC) value (Akaike, 1974) was selected to describe the temporal development of lactate concentration for krill captured in that specific haul. All analyses were performed using the software package R version 3.6.1 (R Core Team, 2013). Fit of each model to the experimental data was tested using the R-function nls for nonlinear least squares estimation because Model 4 is nonlinear. AIC values were obtained using the R-function AIC. For each individual haul, the model with lowest AIC value was selected to model the temporal development of lactate concentration.

In addition to modelling and assessing the temporal development of lactate concentration for krill caught in individual hauls, we also pooled the data and plotted them together to determine whether they followed a similar temporal pattern for lactate development. In case results for individual hauls could be fitted into a similar temporal pattern, an additional analysis as described above was conducted based on pooling data from all hauls.

For krill from the controlled mesh-penetration experiments, we used the same modelling approach as described for the trawl escapees. Finally, 95% Efron confidence intervals (Efron, 1982) were obtained for the mesh-penetration group and the control group results separately using bootstrapping.

Furthermore, to investigate the ability of the lactate measurements to predict escape survival for krill we applied a linear regression analysis between lactate concentration at T_0 for trawl escapees *versus* escape survival after 60 h of onboard monitoring. This analysis was conducted using the R-function ln.

RESULTS

Table 1 summarises the hydrographic data for each trawl haul and catch levels in both cod end and cover. The escape survival column (P_{60}) values for these hauls in Table 1 are from Krafft *et al.* (2016).

Krill in Group 1 had the highest lactate concentrations (Table 2, Fig. 2). Between-haul lactate levels varied from 3 to 7 $mmol^{-1}$ at T_0. When plotted on the same time scale or modelled pooled (Fig. 3), all six hauls followed the same exponential lactate clearance pattern from T_0 towards an asymptotic lower lactate level of ~ 0.3 $mmol^{-1}$. After approximately 200 min, lactate levels were within the 95% confidence limit of the control group (Fig. 3). Table 3 lists the selected models for describing the individual hauls for trawl escapees.

No significant difference in lactate concentrations was found between the simulated mesh penetration and control group. The lactate concentrations for both groups did not exhibit the exponentially decreasing pattern observed for Group 1, and the data were described by constant models with overlapping bootstrapped 95% confidence limits between models (Fig. 4).

We examined the ability of the lactate measurements to predict escape mortality for krill by linear regression between lactate values at T_0 for trawl escapees and escape mortality after 60 h of onboard monitoring. The obtained P value of 0.68 indicated no linier correlation between lactate levels at T_0 and the escape mortality after 60 h (Fig. 5).

DISCUSSION

Results showed that krill had the physiological ability to recover from fishery-induced stress. The lactate clearance time of trawl escapees was well described by an exponential decay function, meaning that the lactate concentration decreased most rapidly near T_0 and thereafter decayed towards a lower asymptotic value that was not significantly different from that of the control group after 200 min. Although krill in the mesh-penetration group demonstrated no measurable signs of stress, net selection as an isolated variable of stress induction may not be sufficient to quantify the actual stress experienced during a natural trawling process. Additional factors, alone or in combination and before and/or after mesh penetration during the capture and handling process, were the main causes of the elevated stress levels observed for the Group 1 individuals, and avoidance behavior during trawling may also play a role.

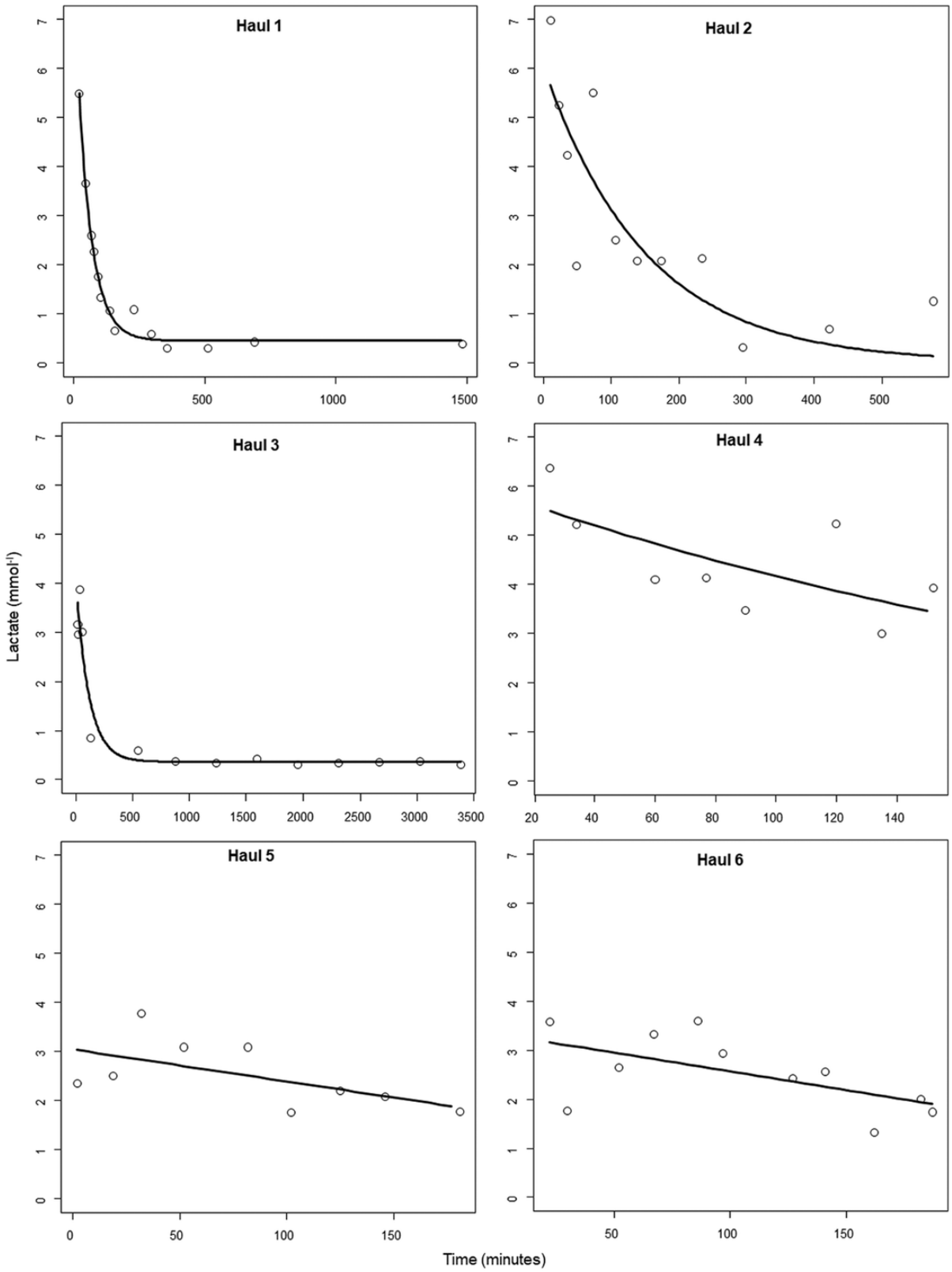

Figure 2. Lactate measurements for trawl escapees (Group 1) of *Euphausia superba* over time for hauls 1–6. Open marks represent single measurements and the black line the mean model of lactate clearance for the single hauls. Notice the different monitoring intervals on the x-axis.

The exponential clearance of lactate occurred quickly, followed by longer and slower clearance towards a lower asymptotic lactate value. Qualitative visual observations of the trawl escapees in the holding facilities showed abrupt swimming behavior during the first 1–2 h, after which normal swimming behavior resumed. Krafft *et al.* (2016) reported average survival probability of 0.96 ± 0.04 (range 0.88–1.00) at 60 h after the trawl arrived on deck for cod-end mesh escapees. This equaled between-haul escape

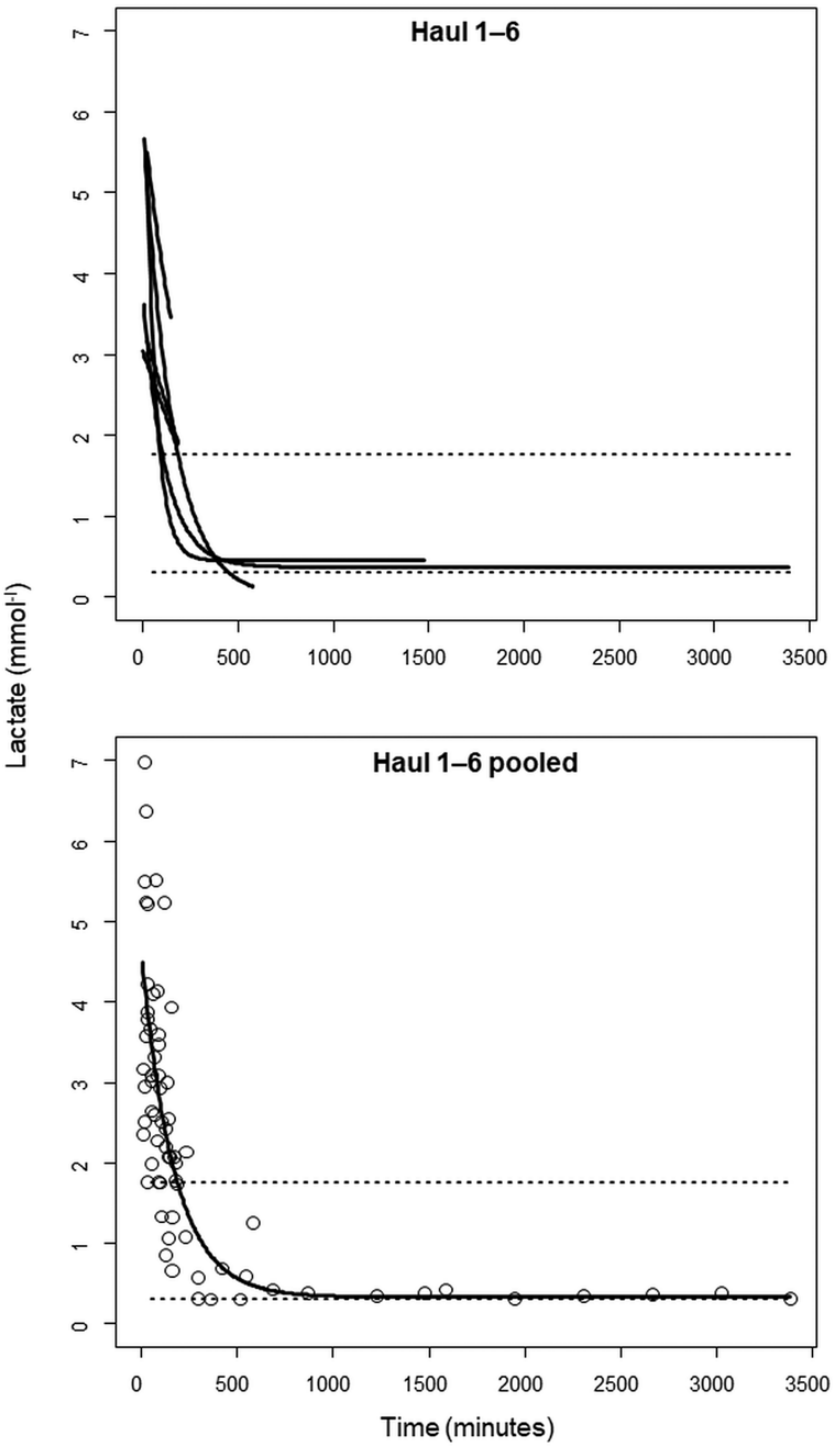

Figure 3. Modelled lactate levels for individual hauls of *Euphausia superba*. Broken lines represent 95% confidence limits for the control (Group 3). The lower panel indicates all hauls pooled and fitted for selected model. The broken lines represent the range of observed lactate levels in the control (Group 3).

mortality ranging from 0 to 12% (average 4.4 ± 4.4%), which suggested that krill were tolerant of the capture-and-escape process in trawls. A 60 h monitoring period was enough time for krill to fully recover from physiological stress. These results further support the validity of findings from on-board experiments with live krill showing that they seem to acclimatise quickly and are relatively robust animals.

Peak lactate levels following stress or exhaustion in crustaceans vary considerably with species and conditions. Trawled swimming crabs (*Liocarcinus depurator* Linnaeus, 1758) not subjected to air exposure had peak lactate levels of 2.43 ± 0.35 $mmol^{-1}$, whereas air exposure increased average hemolymph lactate levels to 6.24 ± 0.22 $mmol^{-1}$ (Bergmann *et al.*, 2001). In the same study, lactate levels of trawled and immersed squat lobster (*Munida rugosa* Leach, 1820) averaged only 0.87 ± 0.08 $mmol^{-1}$, illustrating the interspecific differences in the magnitude of the lactate response. Few reports of hemolymph lactate levels in krill exist, and the maximum lactate response is not known. Tremblay & Abele (2016)

Table 3. AIC values for models 1-4 for individual hauls of *Euphausia superba*; *, model unable to converge. Selected model is indicated as bold AIC value.

Haul	Model 1	Model 2	Model 3	Model 4
1	54.27	51.89	14.47	**−1.46**
2	54.70	48.31	**41.70**	42.23
3	51.00	43.06	24.41	**19.72**
4	27.13	24.96	**24.51**	*
5	21.56	**19.73**	20.04	*
6	28.68	**26.16**	26.61	*

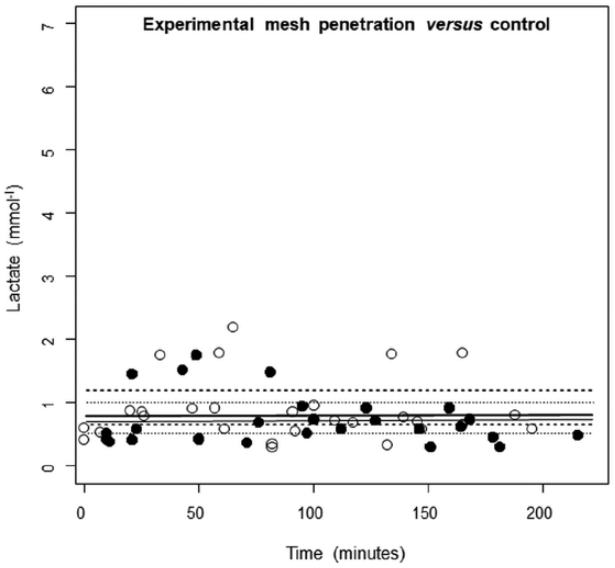

Figure 4. Open marks represent measured lactate levels for controlled mesh penetration (Group 2) and black filled marks are lactate levels for controls (group 3) of *Euphausia superba hauls*. Dotted lines are 95% confidence limits for mean values for the control (Group 3) and broken lines the 95% confidence limits for controlled mesh penetration (Group 2). There is no significant difference in measured lactate levels between Group 2 and Group 3 as confidence limits overlap.

observed hemolymph lactate concentrations of 2.3 ± 0.3 mmol^{-1} in *E. superba* following capture at the surface, which is somewhat lower than the average values observed in our study (4.66 ± 0.77 mmol^{-1}). Tremblay & Abele (2016) also studied the metabolic response of *E. superba* to hypoxia using sealed metabolic chambers in which the individuals created their own hypoxic (and presumably hypercapnic) conditions, reaching an oxygen saturation of ~20% after 12 h and resulting in hemolymph lactate concentrations of 3.5 ± 0.7 mmol^{-1}. In comparison, the Group 1 individuals in the present study had higher lactate levels (3–7 mmol^{-1} at T_0). Duan *et al.* (2014), however, reported that exhaustive swimming by whiteleg shrimp (*Litopenaeus vannamei* Boone, 1931) resulted in peak lactate levels of 9.4 ± 1.6 mmol^{-1}, whereas hypoxia exposure down to ~25% resulted in a more moderate lactate response (6.8 ± 1.0 mmol^{-1}). If exhaustive swimming elicits a greater lactate response, this may explain the lactate levels observed in our study. All examined krill species, including *E. superba*, display avoidance reactions by actively avoiding fishing gear and other threats (O'Brien, 1987). The initial behavior consisted of moving away from the threat using pleopod swimming. As the threat intensifies, krill may use the so-called flash expansion, whereby an initial condensation of the school is following by a dispersal using repetitive

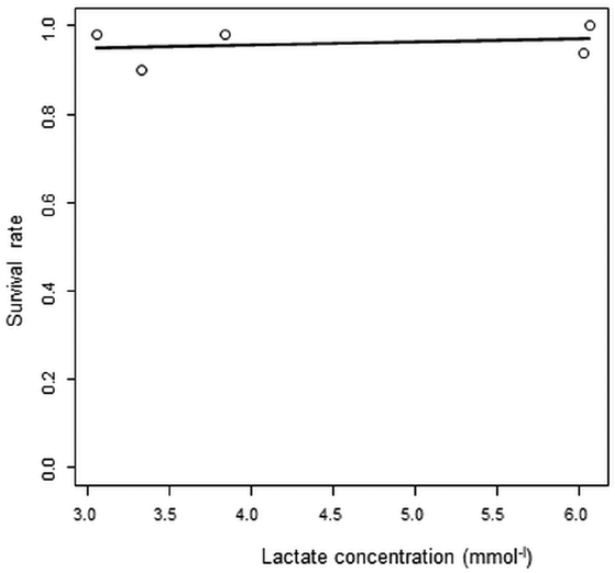

Figure 5. Linear correlation between survival rate of *Euphausia superba* individuals after 60 h from Krafft *et al.* (2016) and lactate concentration at T_0 of trawl escapees. There is no linear correlation between lactate concentration in trawl-caught individuals and survival rate after 60 h.

tail-flip swimming (O'Brien, 1987). In krill, tail-flip swimming can yield high swimming speeds, up to four body lengths per flip (O'Brien, 1987), and data from kuruma shrimp (*Marsupenaeus japonicas* Spence Bate, 1888) suggests that tail-flip swimming can only be sustained for a short duration before exhaustion occurs (Yu *et al.*, 2009), presumably, due to rapid accumulation of hemolymph lactate (Henry *et al.*, 1994; Duan *et al.*, 2014).

The lactate clearance time measured in our study is comparable to findings from other crustaceans, such as trawl-caught and subsequently discarded or otherwise handled Norwegian lobster (*Nephrops norwegicus* Linnaeus, 1758) (Harris & Andrews, 2005), spiny lobster (*Panulirus argus* Latreille, 1804) (Vermeer, 1987), or school prawns (*Metapenaeus macleayi* Haswell, 1879) (Broadhurst & Uhlmann, 2007). Broadhurst & Uhlmann (2007) reported that crustaceans may be more tolerant than other taxa to handling because of their durable exoskeleton. An exoskeleton in combination with fast clearance of lactate make krill relatively robust as trawl escapees and may explain their robustness and low escape mortality rate, as also reported in Krafft *et al.* (2016). Antioxidant levels (e.g., astaxanthin) in krill are especially high, suggesting that they provide benefits against oxidative damage (Tou *et al.*, 2007). Among the various carotenoids, astaxanthin is the most potent known natural antioxidant against oxidative stress (Satoh, 2016). If an individual's escapement from a trawl is successful with a limited number of gear contacts and a single mesh penetration, the current results indicate that krill will experience little physiological impairment and are expected to be in a good physiological state and thus not likely to experience increased post-escape vulnerability (e.g., to predation).

The six hauls we conducted to collect trawl escapees all yielded individuals with elevated levels of lactate in the hemolymph. Although these six hauls contained different numbers of individuals, they were monitored at different time intervals, and were caught under varying conditions in terms of depth and towing time, the lactate clearance rates of krill showed a similar and consistent pattern. This include hauls 5 and 6, which alone were best described by a constant model over their limited sampling intervals, but modelled together with the rest of the hauls is well explained by an exponentially declining function.

Our simulated mesh-penetration experiment tested the effect of a single mesh penetration per individual, whereby the krill were oriented correctly relative to the mesh opening to successfully escape. Krag *et al.* (2014) speculated that krill may be capable of orienting themselves relative to the meshes and thereby escape head-first or they may experience multiple contacts with the netting and at some stage simply by chance meet the mesh opening head-first and escape. If krill escape a trawl with limited netting contact, the escapement process will inflict little or no physiological stress. Under such a scenario, the increased lactate levels measured in Group 1 at T_0 would be more influenced by the methodology used the collect and retrieve escapees than the trawl escapement itself. Retention in the net during trawling, crowding of individuals, haul-back and onboard retrieval of the net are all stressors, that in addition to the mesh penetration in itself, potentially all can contribute to an elevated lactate level observed for Group 1 compared to Group 2. Krill behavior in trawls or the processes that lead to size selection in trawls is nevertheless poorly understood. If the method used to collect trawl escapees contributes to the observed physiological stress levels, the covered cod-end technique may systematically overestimate the stress levels experienced by escapees at depth and most likely also the escape mortality. Stoner (2012a) concluded that the complexity of interactions and rapid changes in the chemical constituents in the hemolymph and other tissues make predicting mortality based on these variables difficult. Our results for trawl caught krill also showed that hemolymph lactate concentrations at T_0 is a poor predictor for escape mortality. Stoner (2012a) suggested the use of behavior-based evaluation of animal vitality and suggested the RAMP (Reflex Action Mortality Predictor) approach to predicting mortality. RAMP is based on reflex-based indices whereby each individual is inspected and assigned a score. For spot prawns (*Pandalus platyceros* J.F. Brandt in von Middendorf, 1851), the RAMP approach was an excellent predictor of delayed mortality (Stoner, 2012b). RAMP, however, was not developed or tested for small pelagic crustaceans such as krill, and its applicability to predicting mortality or post-escape vulnerability of krill is unknown. In contrast to the RAMP approach, which include some subjectivity, point of care devices like the handheld i-STAT will reduce potential observer bias as it produces a numerical value of the physiological impairment.

Difficulties in predicting mortality of escapees based on blood chemistry are not limited to crustaceans, as several commercial fish species show a similar lack of relationship. Stoner (2012a) nevertheless showed that physiological parameters provide insight into the mechanisms of stress responses and help identify critical adaptive metabolic processes in the capture and post-capture processes despite being unrelated to mortality outcomes. Stoner (2012a) further concluded that methods to obtain physiological parameters are difficult and expensive to carry out in routine field or factory applications. Point of care devices such as i-STAT offer a solution, as they facilitate fast and reliable measurements of physiological stress parameters during fieldwork in remote regions such as the Southern Ocean, thereby enabling real-time monitoring of causes and amplitudes of stressors, which can be used to quantify and optimise sampling methodologies and holding systems.

ACKNOWLEDGEMENTS

This study was financed by Havforskningsinstituttet (Institute of Marine Research) in Norway and the Norwegian Research Council as part of the SILF project (grant 243619). We also extend our gratitude to Olympic AS for providing FV *Juvel* and its crew to conduct this survey free of charge. We are most grateful to the captain, officers, and crew on board for logistical support provided during the cruise. We would like to thank the two anonymous reviewers and the Associate Editor for providing valuable suggestions and comments which greatly improved this manuscript.

REFERENCES

Akaike, H. 1974. A new look at the statistical model identification. *IEEE Trans Auto Control*, **19**: 716–722.

Bergmann, M., Taylor, A.C. & Moore, G. 2001. Physiological stress in decapod crustaceans (*Munida rugosa* and *Liocarcinus depurator*) discarded in the Clyde Nephrops fishery. *Journal of Experimental Marine Biology and Ecology*, **259**: 215–229.

Broadhurst, M.K., Suuronen, P. & Hulme, A. 2006. Estimating collateral mortality from towed fishing gear. *Fish and Fisheries*, **7**: 180–218.

Broadhurst, M.K. & Uhlmann, S. 2007. Short-term stress and mortality of juvenile school prawns, *Metapenaeus macleayi*, discarded from seines and trawls. *Fisheries Management and Ecology*, **14**: 353–363.

Brown, J.A., Watson, J., Bourhill, A. & Wall, T. 2008. Evaluation and use of the Lactate Pro, a portable lactate meter, in monitoring the physiological well-being of farmed Atlantic cod (*Gadus morhua*). *Aquaculture*, **285**: 135–140.

Chang, E.S., Sage, B.A. & O'Conner, J.D. 1976. The qualitative and quantitative determinations of ecdysones in the tissues of the crab, *Pachygrapsus crassipes*, following molt induction. *General and Comparative Endocrinology*, **30**: 21–33.

Chang, E.S., Keller, R. & Chang, S.A. 1998. Quantification of crustacean hyperglycemic hormone by ELISA in hemolymph of the lobster, *Homarus americanus* following various stresses. *General and Comparative Endocrinology*, **111**: 359–366.

Dana, J.D. 1850. Synopsis generum crustaceorum ordinis "Schizopoda." *American Journal of Science and Arts*, Series 2, **9**: 129–133.

Duan, Y., Zhang, X., Liu, X. & Dhanrajsingh, N.T. 2014. Effect of dissolved oxygen on swimming ability and physiological response to swimming fatigue of Whiteleg shrimp (*Litopenaeus vannamei*). *Oceanic and Coastal Sea Research*, **13**: 132–140.

Efron, B. 1982. The jackknife, the bootstrap and other resampling plans. SIAM Monograph No. 38, CBSM-NSF. Stanford University, Stanford, CA, USA.

Fanjul-Moles, M.L. 2006. Biochemical and functional aspects of crustacean hyperglycemic hormone in decapod crustaceans: Review and update. *Comparative Biochemistry and Physiology C*, **142**: 390–400.

Gallagher, A.J., Frick, L.H., Bushnell, P.G., Brill, R.W. & Mandelman, J.W. 2010. Blood gas, oxygen saturation, pH, and lactate values in elasmobranch blood measured with a commercially available portable clinical analyzer and standard laboratory instruments. *Journal of Aquatic Animal Health*, **22**: 229–234.

Harris, R.P. & Andrews, M.B. 2005. Physiological changes in the Norway lobster *Nephrops norvegicus* (L.) escaping and discarded from commercial trawls on the West coast of Scotland II. Disturbances in hemolymph respiration gases, tissue metabolites and swimming performance after capture and during recovery. 2005. *Journal of Experimental Marine Biology and Ecology*, **320**: 195–210.

Harter, T.S., Shartau, R.B., Brauner, C.J. & Farrell, A.P. 2014. Validation of the i-STAS system for the analysis of blood parameters in fish. *Conservation Physiology*, **2**: [doi: 10.1093/conphys/cou037].

Henry, R.P., Booth, C.E., Lallier, F.H. & Walsh, P.J. 1994. Post-exercise lactate production and metabolism in three species of aquatic and terrestrial decapod crustaceans. *Journal of Experimental Biology*, **186**: 215–234.

Krafft, B.A., Krag, L.A., Engås, A., Nordrum, S., Bruheim, I. & Herrmann, B. 2016. Quantifying the escape mortality of trawl caught Antarctic krill (*Euphausia superba*). *PLoS ONE*: [doi: 10.1371/journal.pone.0162311].

Krag, L.A., Herrmann, B., Iversen, S.A., Engås, A., Nordrum, S. & Krafft, B.A. 2014. Size selection of Antarctic krill (*Euphausia superba*) in trawls. *PLoS ONE*: [doi: 10.1371/journal.pone.0102168 PMID: 25105960].

Malte, C.L., Jakobsen, S.L. & Wang, T. 2014. A critical evaluation of automated blood gas measurements in comparative respiratory physiology. *Comparative Biochemistry and Physiology A*, **178**: 7–17.

Morris, S., Postel, U., Mrinalini, Turner, L.M., Palmer, J. & Webster, J.G. 2010. The adaptive significance of crustacean hyperglycaemic hormone (CHH) in daily and seasonal migratory activities of the Christmas Island red crab *Gecarcoidea natalis*. *Journal of Experimental Biology*, **213**: 3062–3073.

O'Brien, D.P. 1987. Description of escape responses of krill (Crustacea: Euphausiacea), with particular reference to swarming behavior and the size and proximity of the predator. *Journal of Crustacean Biology*, **7**: 449–457.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria [http://www.R-project.org/].

Ryer, C.H. 2004. Laboratory evidence for behaviour impairment of fish escaping trawls: A review. *ICES Journal of Marine Science*, **61**: 1157–1164.

Satoh, T. 2016. Astaxanthin: Health benefits and toxicity. In: *Nutraceuticals: Efficacy, safety and toxicity* (R.C. Gupta, ed.), pp. 531–539. Elsevier, Amsterdam.

Serra-Llinares, R.M., Tveiten, H., Damsgård, B. & Aas-Hansen, O. 2012. Evaluation of a fast and simple method for measuring plasma lactate levels in Atlantic cod, *Gadus morhua* (L.). *International Journal of Fisheries and Aquaculture*, **4**: 217–220.

Stoner, A.W. 2012a. Assessing stress and predicting mortality in economically significant crustaceans. *Reviews in Fisheries Science*, **20**: 111–135.

Stoner, A.W. 2012b. Evaluating vitality and predicting mortality in spot prawn, *Pandalus platyceros*, using reflex behaviors. *Fisheries Research*, **119**: 108–114.

Stoot, L.J., Cairns, N.A., Cull, F., Taylor, J.J., Jeffrey, J.D., Morin, F., Mandelman, J.W., Clark, T.D. & Cooke, S.J. 2014. Use of portable blood physiology point-of-care devices for basic and applied research on vertebrates: a review. *Conservation Physiology*, **2**(1): [doi: 10.1093/conphys/cou011].

Tou, J.C., Jaczynski, J. & Chen, Y.C. 2007. Krill for human consumption: Nutritional value and potential health benefits. *Nutrition Reviews*, **65**: 63–77.

Tremblay, N. & Abele, D. 2016. Response of three krill species to hypoxia and warming: an experimental approach to oxygen minimum zones expansion in coastal ecosystems. *Marine Ecology*, **37**: 179–199.

Vermeer, G.K. 1987. Effects of air exposure on desiccation rate, Hemolymph chemistry, and escape behavior of the spiny lobster, *Panulirus argus*. *Fishery Bulletin*, **85**: 45–52.

Wileman, D., Ferro, R.S.T., Fonteyne, R. & Millar, R.B. (eds.). 1996. *Manual of methods of measuring the selectivity of towed fishing gears.* ICES Cooperative Research Report, No. 215. ICES, Copenhagen, Denmark.

Yu, X., Zhang, X., Zhang, P. & Yu, C. 2009. Critical swimming speed, tail-flip speed and physiological response to exercise fatigue in kuruma shrimp, *Marsupenaeus japonicus*. *Comparative Biochemistry and Physiology A*, **153**: 120–124.