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The effect of transportation and re-watering strategies on the survival, physiology and batch weight of the blue mussel, *Mytilus edulis*

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Abstract

This study compared conventional and experimental strategies, along a mussel supply chain, before (pre), during and after (post) transportation at pilot commercial scale. The main focus was on depuration and re-watering (immersion of mussels pre-transport and re-immersion at post-transport) and the use of ice versus ambient temperature (0°C and 8°C), and humidity (use of a lid or cover) during transport. Improvements to the supply chain were measured via survival, batch weight dynamics and ammonium serum concentration (as a simple physiological stress assay). We found that ice is not the key management component for keeping mussels alive. Survival and stress were reduced via depuration and rewatering. We also present and discuss transport measures strategies benefitting both producers and shellfish merchants, representing a total cost saving of 14 %.

1. Introduction

Blue mussels, *Mytilus edulis*, are ubiquitous across N. Atlantic coasts; the main producer countries lie in Western Europe, Canada and the USA (FAO, 2014).

An international trade has been established, given the value of the subsector (*ca.* $385 million) and volume (aquaculture production over 184,000 tonnes; data from 2012, FISHSTAT, 2014). The trade in "live" product is popular with air, sea and road networks enabling transportation of bulk volumes of live shellfish (FAO, 2014).

Particular supply chains may differ from harvest to point of sale, although the following procedures are generally typical: rope grown mussels are removed from longlines and are washed, and de-clumped from each other and fouling organisms, before being "graded" according to size. Depending on the bacterial loading of the original water body and national legislation, mussels may also be "depurated" (immersed in clean seawater, for up to 42 h, to promote removal of bacteria), and then de-byssed (byssal threads removed). Onward transportation may be up to 48 h duration. On arrival, mussels can be sold immediately, maintained in cold, damp storage, or immersed again ("re-watered" or "re-conditioned") to improve quality prior to sale Lee *et al*., 2008; Wyatt *et al*., 2013). The term "re-watering" will be used in this study to define immersion of mussels in seawater either at pre-transport stage or later in the supply chain at a post-transport stage.

M. edulis demand immersion to enable excretion of ammonia, aerobic respiration and feeding but as an intertidal bivalve, it can tolerate periods of emersion and temperature variation for extended periods by closing both shells via an adductor muscle. During prolonged hypoxic events the mussel will open the shell (gape) to breathe air, can depress metabolic rate and respire anaerobically; haemolymph ammonium concentration also increases during emersion (Sadok *et al*.,, 1999). For rope grown mussels, the effects of emersion are compounded since the animals

are constantly immersed until harvest. The adductor muscle is usually smaller, and shell less dense compared to bottom grown mussels (Christensen *et al*., 2012), and they gape during transport. As a consequence, mussels become dehydrated causing a disparity between the weight packed and that received by the wholesaler, leading to disputes (Barrento *et al*., 2013).

The live trade of shellfish relies on both quantity (i.e., high survival) and product quality (e.g. fresh smell). To improve the industry it is essential to understand the ecology and physiology of *M. edulis* and relate it to commercial practices and economic demand particularly for the rope grown mussel sub-sector (Barrento *et al*., 2013a; Barrento, *et al*., 2013b; Gallardi *et al*., 2014). In this study we compared husbandry practices, at pilot commercial scale, for rope grown *M. edulis* before, during and after transport, in order to inform and improve the quality, quantity and efficiency.

2. Materials and Methods

2.1 Animals, transportation, aquarium systems and experimental design.

Rope grown blue mussels, *Mytilus edulis*, mean length 63.3 ± 7.1mm were obtained from Muckairn Mussels Ltd (Argyll, UK). Sampling occurred on the batch over a number of stages, during a 4 day simulated supply chain (see Fig. 1) from Argyll to the Centre of Sustainable Aquatic Research (CSAR), Swansea, UK.

Mussels were initially sampled on site immediately, de-clumped and graded (Figure 1). The batch was then split into two on site, with half receiving depuration or storage on ice (i.e., non-depurated). After 48h duration, further pre-transportation sampling occurred. Depurated and ice stored mussels were then transported by road (total emersion time, 22h duration) to CSAR in multiples of commercial mesh bags (weight *ca.* 5 kg). Bags were contained in polystyrene boxes

under a matrix of an additional four treatments: with ice at 0°C and at ambient chilled conditions of ca. 5°C; and with different humidity, i.e. with or without a lid.

On arrival, post-transport sampling occurred on random bags within specific treatments, which were then removed from the experiment. Remaining bags were then placed into 1,500L circular tanks within a 10,000L recirculating aquaculture system (RAS at 7°C) to re-water the mussels for 24h. The tanks were flushed copiously (i.e., water sent to waste) for the first 2h, with a hydraulic retention time of *ca.* 2h thereafter. RAS water treatment included sand filtration, protein skimmers, bacterial bio-filters and UV sterilisation. After a further 22h, further bags within treatments were sampled (post re-watering) for a final time.

An additional experiment investigated post-transport weight gain of mussels, i.e. upon re-watering, over a 1h period. Mussel bags were weighed after transportation and immersed in RAS at 5 or 10°C for 10, 20, 30 and 60 min before reweighing.

2.2. Parameters and assay measurements.

Serum ammonium (*n*=30), was sampled at all stages, but for post-transportation was restricted to treatments with lids (i.e., Ice&Lid or Lid only, for both depurated and non-depurated fractions only). Each individual sampled was considered a replicate. Haemolymph was removed from the adductor muscle using a 21 gauge needle and syringe, and immediately placed on ice. Samples were centrifuged (10,000 x *g*; 5min) on a portable microcentrifuge (Galaxy, VWR International LLC, USA) with 100 µL aliquots frozen until further use. The ammonium assay was performed with modifications on the Indophenol method after Bolz and Howel (1978) with modifications for a 96 well plate. Serum was defrosted and deproteinised with an equal amount of 5% TCA. After further centrifugation, 100 µL of supernatant was diluted 5-20 fold in 3% sodium chloride

solution. To 100 µl diluted serum in triplicate, 50 µL of reagent 1 (phenol 1g; sodium nitroprusside 5mg; 50mL ddH_2O) was added and shaken for 30s; 50 µL of reagent 2 (sodium hydroxide 0.5 g; sodium hypochlorite 1 mL; 50ml ddH_2O) were then added and the plate was incubated at 37°C for 30 min. Optical density was determined at 635nm spectrophotometer (Sunrise, Tecan Group Ltd, Switzerland). Ammonium content of the serum samples were then calibrated against a standard curve of ammonium chloride (0 – 1 µg/mL).

For survival and mortality, individual mussels were extracted or removed from bags after grading, depuration and transport (n=100) and 24h after re-watering (n=300). and checked for gaping and death (defined as lack of adductor muscle contraction when tapped, or shells broken due to physical trauma). Even though mussels with broken shells are not dead, they are discarded because according to the quality criteria used by the industry, these mussels are therefore worthless. Each individual sampled was considered a replicate. Changes in biomass (difference in mass, post-transport or post-rewatering) were calculated as a batch weight (*n*=4-8 bags of mussels) weighed on a bench scale (Ohaus Europe GmbH, Switzerland).

2.3. Statistics. Data were analysed using GraphPad-Prism (GraphPad Software, San Diego, USA). All data shown are mean±1 SEM and were tested for normality and homogeneity of variances. Data were compared between treatments using appropriate parametric statistics of normally distributed or transformed data, including arcsin data for percentage values. For survival/mortality, percentage values are displayed but Fisher's Exact Test was used to compare absolute numbers of live and dead mussels between treatments. For batch weight, percentage differences are displayed but probits (arcsin percentage values) were used for student's *t*-test and one-way ANOVA. Finally for serum ammonium, one-way ANOVA was used to compare values within discrete stages of the supply chain. ANOVA was followed by Tukey post-hoc test.

3. Results

3.1. Mortality

Mortality varied from 2 to 7% throughout the different stages of the supply chain (Fig. 2A). At the pre-transport stage mortality was low (3% after grading; 2% after 48h depuration). Non-depurated mussels had approximately 3 times the mortality rate (6%) compared to depurated counterparts, although this was not statistically significant. Depuration or transport conditions did not affect post transport mortality (*ca.* 4% for all treatments). However at the post-rewatering stage, mussels in the non-depurated, ambient treatment did increase mortality compared to the other treatments (*ca*. 7% compared to 1-3%). This was significantly higher than mussel mortality in the depurated, iced treatment (Fisher's Exact, $P<0.05$-0.01).

3.2. Ammonium

Serum ammonium concentrations ranged between *ca.* 220 and 830 μM, with generally lower concentrations found in the depurated treatments at the pre- and post-rewatering stage (Fig. 2B). After the grading process, mussel serum ammonia was 311μM. This decreased significantly for mussels in the depurated treatment and increased significantly for non-depurated mussels (238 and 500 μM respectively; ANOVA, $P<0.05$). Post-transport, serum ammonium remained low for depurated mussels (under 555μM) whilst in comparison, non-depurated mussels experienced a significant increase in serum ammonia irrespective of transport temperature (688-838 μM; ANOVA, $P<0.05$-0.001). Mussels transported on ice showed a trend for increased ammonium concentration compared to their non-iced counterparts; for depurated mussels, this trend was significant (ANOVA, $P<0.01$). Post-rewatering serum ammonium concentrations fell to under *ca.* 330 μM, not significantly different between treatments.

3.3. Weight Loss

Pre- and post-transport treatments showed clear trends for changes in batch weight (Fig.3A). All non-depurated mussels lost between 13.4-16.7% mass, irrespective of transport conditions, and were significantly different to all depurated mussels (ANOVA, $P<0.05$-0.001). Depurated mussels lost between *ca.*2-8% mass, with the iced and lidded treatment (2.7%) significantly lower than mussels transported under ambient and unlidded conditions (8.2%; ANOVA, $P<0.01$).

Re-watered mussels regained batch weight very quickly upon immersion in water regardless of temperature (Fig 3B). After only 10 min, mussel mass increased to 13.7 and 15.7% and after 60 min immersion to 17.8 and 17.1% at 5 and 10°C, respectively. There were no significant differences in mass increase between temperature regimes, or between immersion times at 10°C. However, at 5°C, the batch weight gain after 30 and 60 min was significantly greater than after 10 min (ANOVA, $P<0.05$).

4. Discussion

This study is the first to our knowledge that examined mussels' physiology (ammonium concentration in the serum, weight changes and survival) along the supply chain from a broad approach perspective by following all steps of the supply chain: grading, depuration, transport and re-watering after transport. Previous studies by Harding *et al*. (2004a) examined post-harvest stress response in *M. edulis* during rewatering, iced, or chilled conditions. However, this study took a broader value chain approach to further include pre- and post-transport conditions. Mussels lose weight when out of water which is a consequence of gaping and loss of mantle cavity water (Bayne, 2009). Interviews made in Scotland to mussel farmers and dispatchers revealed that it is common practice to pack mussels after depuration in net bags (5 kg) but with an excess of weight (usually 5.8 kg per bag) to compensate for circa 8% weight loss, similar to a

Baker's dozen strategy, which we have termed "overpacking" (Barrento *et al*., 2013b). Overall, in this study, depurated mussels lost less weight during transport (3%) than the numbers reported by mussel farmers (8 %). Water loss can vary significantly depending on mussel physiological condition and season; the results we obtained concern early spring only in the North East Atlantic. Most importantly this weight can be quickly re-gained - within 10 minutes - if after transport mussels are re-watered at 10° C. This can be an important strategy to the industry with the advantage that chilling is unnecessary as ambient seawater is more likely to be at 10°C in the NE Atlantic, during this time of year. However further studies, should include seasonality differences, and investigate optimal temperature especially during late summer when temperature increases to a maximum of 15-16°C.

Although weight loss directly affects product quality, it can also lead to mortality and reduction in product quantity. Mussels begin to suffer significant mortality after 20 % or more of body weight lost through desiccation (Bayne *et al*., 1976; Bayne, 1976). In the current study, such a high mortality was not obtained even after a total of 66h out of water (16% cumulative mortality); but air exposure most probably compromised the endurance of non-depurated mussels along the supply chain. Re-immersion following post harvesting (i.e. washing, de-clumping and grading processes) can reduce the impact of physical stressors (Harding, *et al*., 2004a) and increase survival by over 30% or 50% after 12 and 48h, respectively (Prochazka and Griffiths, 1991; Slabyj and Hinkle, 1976). In this study, re-immersion post-harvesting improved the survival of mussels by 33%.

Measurements of ammonium in blood serum provide a rapid, quantitative index of the physiological state of *M. edulis* (Sadok *et al*., 1995). In general, serum ammonium concentration increased after stressful husbandry strategies - mechanical stress and air exposure during harvesting, grading, storage and transport – and decreased whenever mussels were re-watered. For intertidal mussels, ammonia accumulates in the haemolymph during low tide, which indicates that catabolism of nitrogenous substrates continues even when mussels are out of the water (Gosling, 2003; Thompson *et al*., 1978). In this case, ammonium accumulation values are within the natural seasonal range of 171.93 to 992.00 μM NH4-N given for intertidal *M. edulis* (Bayne, 1976; Bayne and Scullard, 1977). There are no known seasonal ammonium values for rope grown mussels, but for the majority of the supply chain it did not compromise mussel survival - ammonium peaks post-transport did not correlate with mortality at discrete stages. In fact, *M. edulis* is capable of tolerating very high haemolymph ammonia levels for extended periods of time (Sadok *et al*., 1995). However, it is interesting to note that the highest mortality recorded was observed post-re-watering when ammonium serum concentrations were reduced; this mortality also occurred for non-depurated mussels transported at 5°C without a lid. This suggests a potential lag with regard to the measured effects of potential physiological damage and observed mortality; that a number of husbandry regimes may impact on the survival of mussels; and that re-watering even for 48h may not safeguard mussels that have experienced prior emersion stress.

In this study we did not perform organoleptic analyses – namely using taste, sight, smell touch to assess quality - but it was clear that mussels re-immersed after transport filtered out accumulated ammonia, trapped mud and sediment, and had a general fresher smell afterwards, and regained lost weight which should correlate with a juicier texture, and thus quality. This is in accordance with previous studies reporting that the condition and quality of mussels can be improved with

re-immersion as liquor is recovered, byssal damage is repaired, and trapped mud can be filtered out of the mussels (Slabyj and Hinkle, 1976; Warwick, 1984).

In addition to testing conventional commercial husbandry practices (re-immersion, chilling) this study also focused on different transport options including the use of ice (recommended by good practice codes; (C-ASD, 2003; Macnamara and Pollock, 1988; SEAFISH, 1997; Seccombe, 1999). We showed that the use of ice and lid prevented weight loss of depurated mussels during 22h transport in comparison to neither ice nor lid. This is most probably the combined effect of lid and ice promoting an even and constant levels of humidity, cold environment and oxygen, which decreases mussels' metabolism and benefits the mussel capacity of acquiring oxygen when out of water, through gaping (Sadok *et al*., 1999). Surprisingly, ice alone did not promote an obvious benefit on mussel survival and ammonium concentration in the haemolymph (anticipated to reduce metabolic rate, and hence the accumulation of metabolites). Most studies reported that wet storage of mussels in ambient temperature water resulted in the lowest stress response (C-ASD, 2003; Chandurvelan *et al*.,Glover, 2013;Chandurvelan *et al*., 2013; Harding *et al*., 2004b; Sadok *et al*., 2003). Melting ice keeps the mussels damp and is used by the industry because it is more practical and less expensive than wet transport and likely reduces proliferation of spoilage bacteria, although this was not investigated. This current study was performed on a calm day in beginning of March when water temperature is at its coldest around the UK, and prior to maturation of mussel gonads and the onset of spawning. The benefits of ice would likely be more apparent on windy, hot days over the summer and beginning of autumn, factors that can trigger spawning, promote desiccation and proliferation of spoilage bacteria.

Overall, these study findings suggest that industry resources should be prioritised toward re-watering prior to transport – this not only promotes freshness of the product, but could prevent up to 10% weight loss during emersed transport and reduce mortality by up to 4%. Adoption of these transport strategies could therefore reduce overpacking within the industry, benefitting both producers and shellfish merchants, representing a total cost saving of 14 %.

Re-watering after transport also appears to improve weight gain rapidly (up to 16% in 10 minutes) and in the longer term, reduce mortality and serum ammonium concentration and potentially increasing perceived product quality. This should be included in good practice codes and European legislation provided seawater quality (i.e. free of contaminants and bacteriological load) is guaranteed.

Future research should focus on re-watering from different perspectives: seasonality differences, implementation feasibility (i.e. duration of re-watering), physiological tolerance and overall quality. Transport experiments could further investigate oxygen saturation and humidity using appropriate probes or loggers. Implementation feasibility studies need to include economic benefits, political framework and training needs of authorities and producers alike.

Figure Legends

Figure 1. Experimental design diagram. Weight and mortality was checked after all supply chain stages considered in this study (Grading, Depuration, Transport and Re-watering) for both depurated and non-depurated batches. Haemolymph post-transport was only sampled from mussels of two treatments – Ice & Lid and Lid only.

Figure 2. Mussel performance along supply chain (pre-transport, post-transport and post re-watering stage, analysis performed discretely within stage). **A.** Percentage survival of mussels,, *n*=100 for mussels after grading, depuration and transport) n=300 for mussels after re-watering. Analysis performed on absolute numbers of dead vs live mussels, **statistically significant at P<0.01%. **B.** Serum ammonium concentration only for treatments with Ice&Lid and Lid only. One-way ANOVA, *n*=30, data shown are mean ± 1SEM. different numbers denote statistical significance at least P<0.05%, inside discrete supply chain stage.

Figure 3. Batch (bag) weight dynamics after transport and brief re-watering regimes. **A.** Average weight loss according to pre-transport husbandry and transport treatments. Percentage weight loss converted to arcsin prior to ANOVA; different letters illustrate significantly different mean weight loss between treatments at minimum P<0.05; *n*=6 bags, data shown are mean percentage ± 1 SEM.**B.** Average weight gain of transported mussels following re-watering under two different temperature regimes and duration; percentage weight loss converted to arcsin prior to analysis; ANOVA within temperature regime and students *t* test between temperatures of identical rewatering duration, *n*=4-6 bags. Data shown as actual percentage ± 1SEM; * significantly different to 10 minute duration at 5°C;*n*=4-8 bags, data shown ± SEM

4. Acknowledgements

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Figure 1

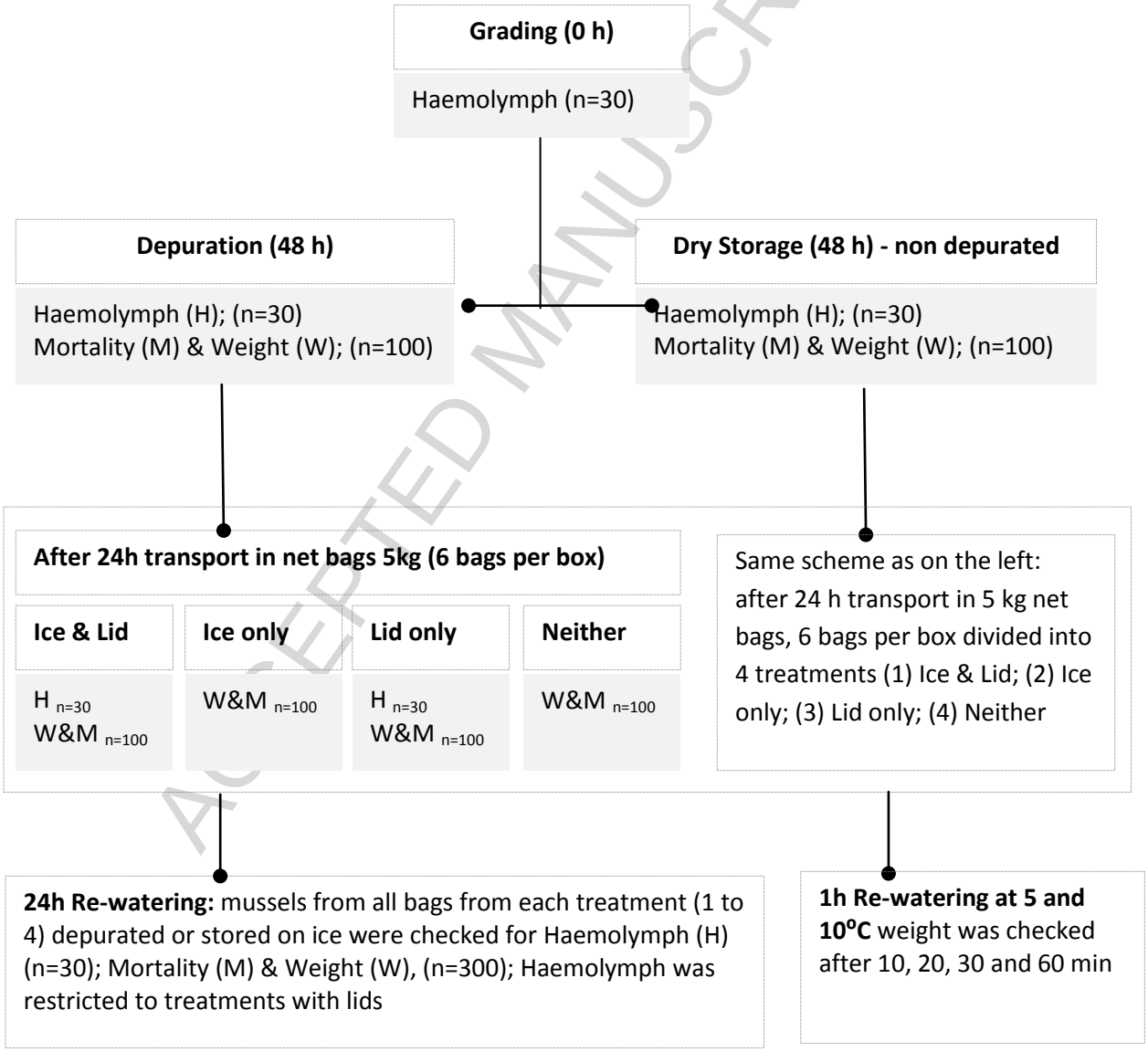
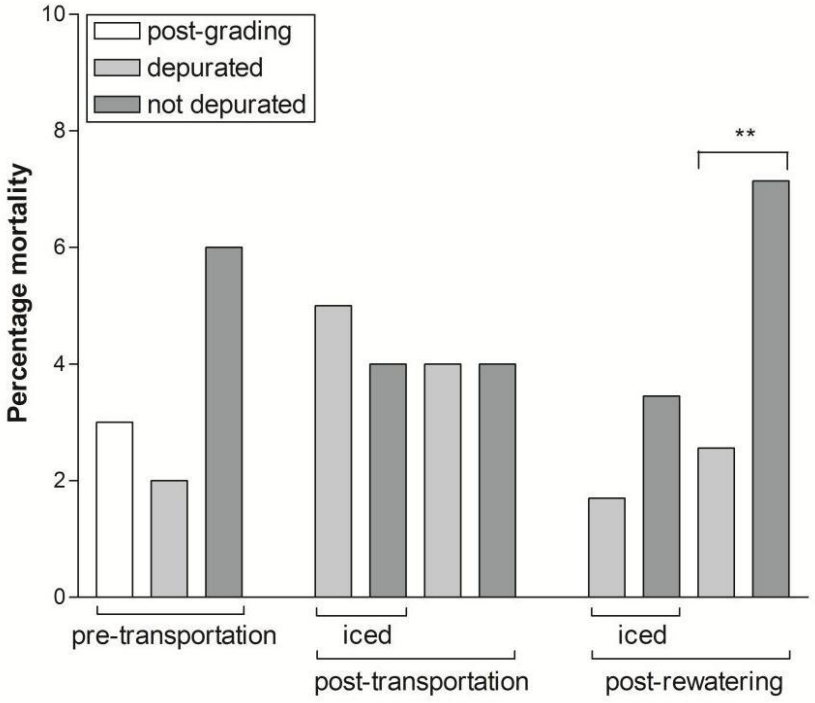


Figure 2

2A



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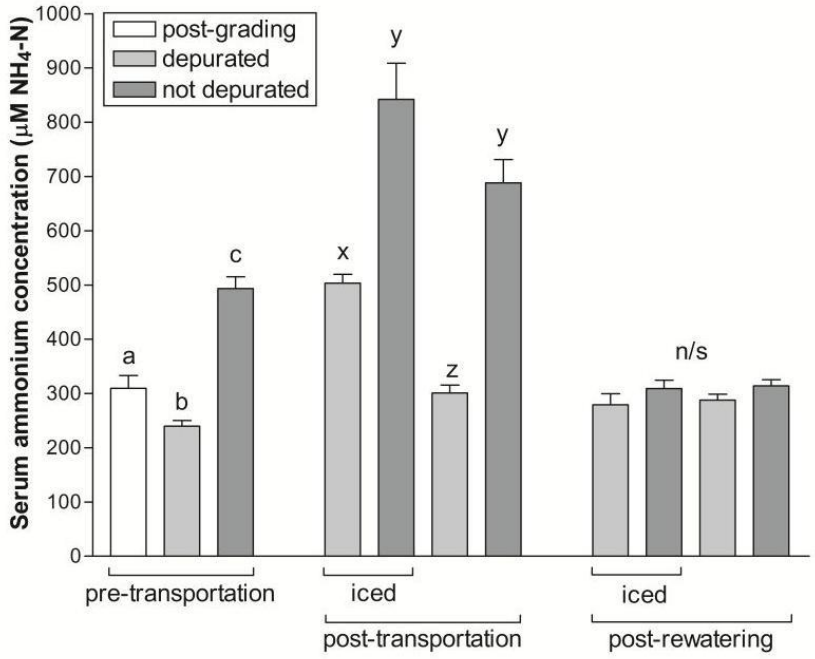
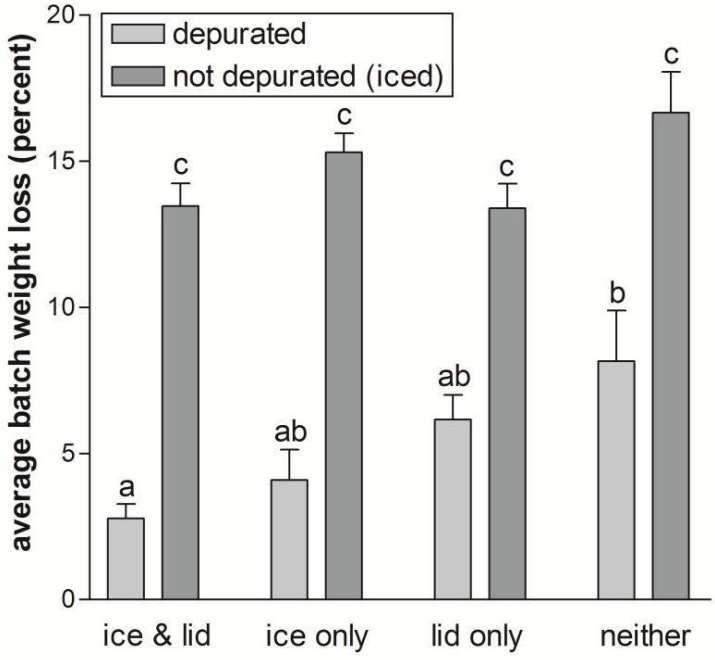


Figure 3

3A



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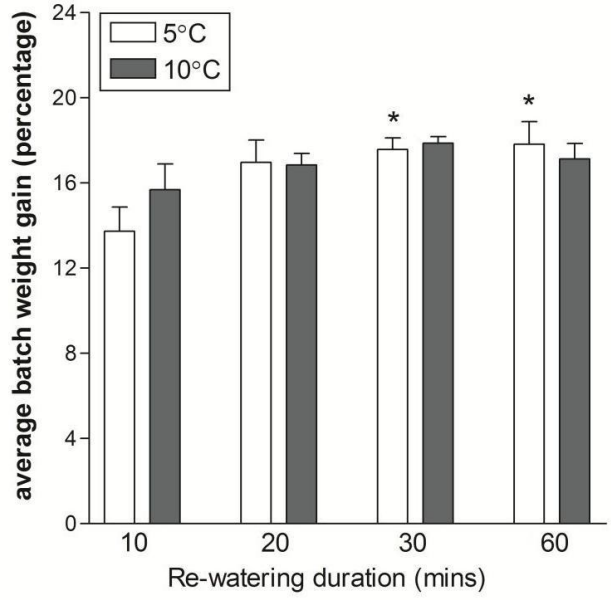

Highlights for review:

1. We followed live mussels trade chain from harvest, depuration, pre and post transport
2. We found that ice is not the key management component for keeping mussels alive
3. Survival and stress were reduced via depuration and rewatering rather than using ice
4. Management and regulation consideration are discussed
5. We stress the importance of academic-industry research leading to better management of mussels