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Short communication

Temperature-dependent morbidity of 'nicked' edible crab, *Cancer pagurus*

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ABSTRACT

The combined effect(s) of holding temperature and claw immobilisation (nicking) on *Cancer pagurus* were investigated. Creel captured animals ($n = 48$) were maintained at 4 °C, 8 °C and 12 °C for 14 days in order to mimic environmental conditions in commercial holding facilities. The consequences of nicking on animal health were assessed by measuring physiological and immune-related parameters in the haemolymph: total protein concentration, L-lactate, pH, haemocyte counts and phenoloxidase activities.

Mortality was most severe in nicked crabs held at 12 °C (83%) compared to non-nicked crabs held at the same temperature (16.7%) or nicked crabs held at lower temperatures (16.7% at 8 °C and 0% at 4 °C). Stress-related parameters such as L-lactate and pH were only affected in the most extreme condition (crabs nicked at 12 °C). However, phenoloxidase activities increased significantly (even in control groups) with increasing temperature, an effect that was exacerbated by the nicking process.

1. Introduction

The brown crab, *Cancer pagurus*, is a commercially exploited shellfish species in Western Europe, with over 60,000 t landed annually. In the UK and Ireland combined, *C. pagurus* fisheries are valued at ~£50 million (Marine Management Organisation, 2013; The Marine Institute and Bord Iascaigh Mhara 2014). Due to the claw morphology of *C. pagurus*, standard elastic and wire banding techniques are unsuitable for preventing the crab from inflicting injuries on conspecifics whilst in captivity. Therefore, nicking or claw mutilation is performed, whereby the apodeme tendons of the chelae are severed (Jacklin and Combes, 2007; Welsh et al., 2013). Routinely, crabs may be held in storage for up to 14 days post-harvest before they are transported to markets. Throughout this entire post-harvest period the shellfish will be exposed to a battery of abiotic stressors, including handling, emersion, temperature fluctuations, overcrowding and food deprivation (Barrento et al., 2008; Albalat et al., 2009, 2010). Such stressors can leave captive shellfish in a weakened state, thereby offering potential pathogens and/or underlying microbes the chance to cause severe infection, spoilage or death (Coates et al., 2012; Shinn et al., 2015).

Welsh et al. (2013) reported higher mortality, an increased number of pathologies and more muscle necrosis in nicked crabs held for seven days in a system in which the temperature ranged between 12 and 15 °C (but was not regulated). This suggests that the condition of the crabs was compromised due to claw nicking (and possibly temperature) by affecting the immune capacity of the animals. Therefore, in the present study, we evaluated the impact of particular holding temperatures on both the physiological condition and the immune-competence of captive *C. pagurus* post-nicking.

2. Materials and methods

2.1. C. pagurus and holding conditions

In February 2014 male *C. pagurus* (42 in total) were collected directly from a commercial creel fisherman fishing off the coast of

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St Abbs, Berwickshire on the east coast of Scotland. At the Marine Station, a sub-set of 36 crabs were placed randomly into one of three tanks (1.4 m^2 floor area, 744 L volume) containing ambient seawater (7–8 °C). Fresh seawater was supplied continuously from below the low-water mark in the adjacent St Abbs Bay. Tanks were divided into two equal chambers using netting that was supported by a fibre glass frame. Each tank contained 12 crabs (6 in each chamber) resulting in a stocking density of 7.51 kg m^{-2}. Over a period of 72 h the temperature in each tank was adjusted from ambient (7–8 °C) to 4 °C, 8 °C or 12 °C. The respective temperatures were maintained for the duration of the experiment (14 days) using heat exchange panels in-built to each tank (Suppl. Fig. 1). Continuous measures indicated that the tank temperatures were maintained at 4.26 °C ± 0.18, 8.07 °C ± 0.20 and 11.68 °C ± 0.37, respectively. Water quality properties including ammonia, nitrate/nitrite and salinity were also monitored for the duration of the experiment.

2.2. Haemolymph extraction and claw immobilisation (nicking)

Following acclimation (72 h), crabs were weighed, tagged and their carapace width was recorded. Haemolymph was then removed from each animal through the sinus at the base of the 5th pereopod using a 25-gauge needle. In total, 1 mL of haemolymph was withdrawn per crab.

After the initial haemolymph samples were taken (time = 0), 6 crabs per tank were nicked using the 'French' method (Haefner, 1971). Briefly, each claw was held open and the apodeme between the upper and lower dactyl of the chelae was severed (Suppl. Fig. 2). After nicking, crabs were returned immediately to their tanks and immersed in sea water to promote clot formation (recommended by Jacklin and Combes (2007)). The crabs were then held for a period of 14 days at the three respective temperatures, and during this time they were not fed, in order to mimic standard industry practices.

To assess the short term (0–4 h) haematological responses to nicking, the remaining 6 males and a further 6 female brown crabs were placed in a tank with ambient (7–8 °C) unfiltered seawater. Three crabs from both sexes were nicked (as described in Section 2.2) and the other animals served as controls. Haemolymph (300 μL) was removed prior to nicking (as described above) and subsequently at 2 h and 4 h post nicking.

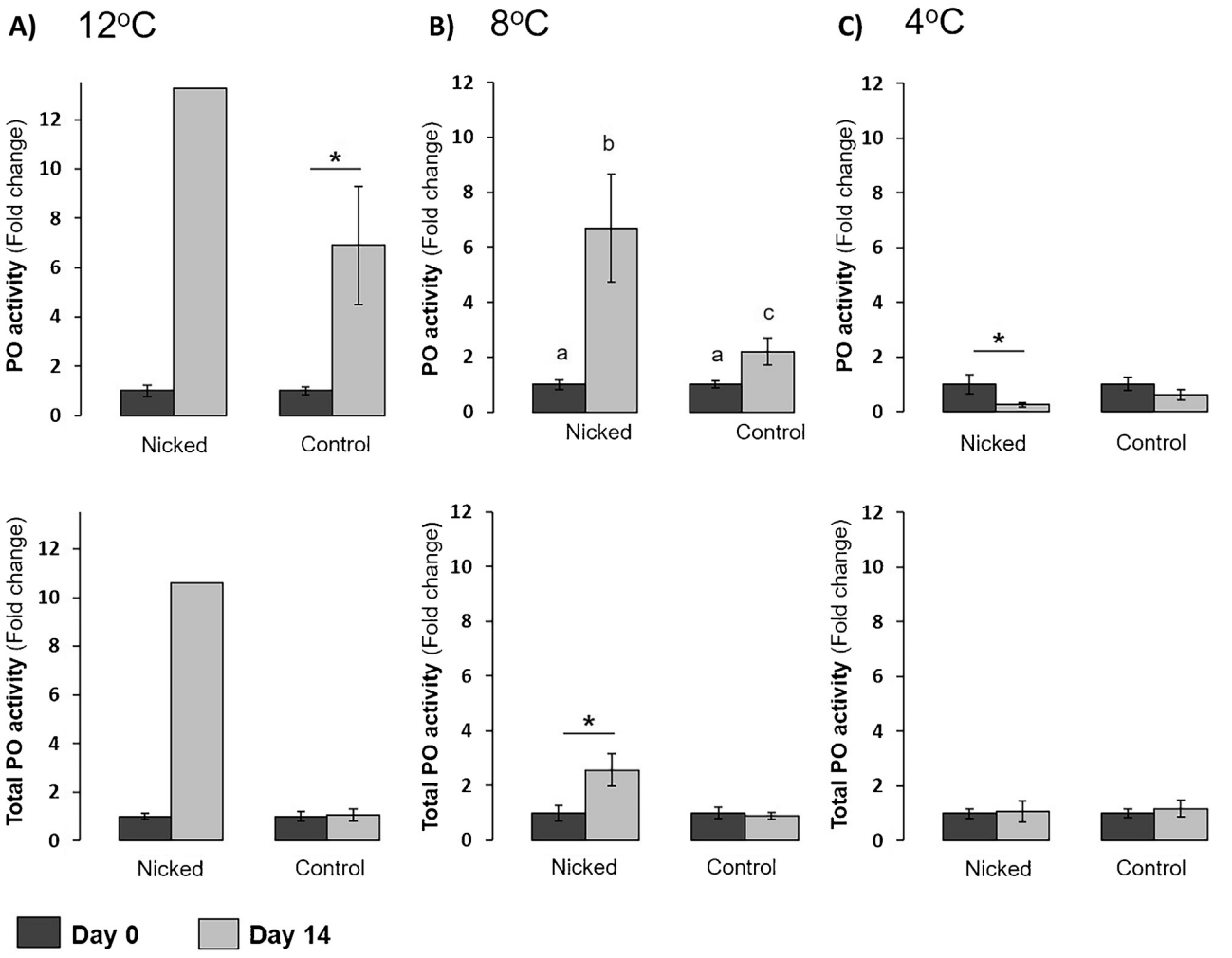

Fig. 1. Phenoloxidase activities within the haemolymph of nicked and control *Cancer pagurus* over the temperature range, 4–12 °C. Active PO was measured in 100 mM Tris–HCl, pH 7.5 using 2 mM dopamine hydrochloride (substrate). Total PO activity (including proPO and haemocyanin-derived PO) was measured by pre-incubating haemolymph samples with 0.1% SDS (3.5 mM) for 5 min prior to the addition of substrate. The formation of product (dopachrome) was monitored at 475 nm. Bar charts illustrate the fold changes in PO activity at day 14 compared to base-line activity measurements recorded on day 0. Values represent the mean ± SD. Unshared letters and * indicate significant differences ($p < 0.05$; $n = 36$). It should be noted that only one nicked crab was alive by day 14 at 12 °C.