Vol. 126: 229–238, 2017
https://doi.org/10.3354/dao03176

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published November 21

Immune status of the spiny lobster *Jasus edwardsii* with tail fan necrosis

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ABSTRACT: Tail fan necrosis (TFN), a disorder commonly found in some populations of commercially fished and cultured lobsters, is thought to be initiated by injuries caused by handling and containment. The unsightly appearance of affected lobster tails significantly lowers their commercial value. Knowledge about TFN is limited. In this study we describe the morphological features of TFN and apply 6 common methods for evaluating the immune status of wild-caught Australasian red spiny lobsters *Jasus edwardsii* with and without TFN. The disease was more frequent in uropods than in telsons of the tail fan, and more extensive on the ventral versus the dorsal surfaces of the tail fan. Missing appendages (i.e. antenna, pereiopod or pleopod) were significantly more common and greater in number for individual lobsters affected with TFN versus those without, possibly as a result of handling in the fishery or as an indirect effect of the disease. Two immune parameters, total haemocyte count and phenoloxidase activity in the haemocyte lysate supernatant (HLS), were significantly compromised in lobsters with TFN. No differences were found in the other immune parameters, i.e. haemocyte viability, haemolymph bacterial count and the protein content of haemolymph plasma and HLS. The results are consistent with injury sustained during prior capture and handling that initiates TFN in these natural caught lobsters. These results raise some potential concerns about the fitness of lobsters in natural populations that are affected by TFN, and some potential solutions are proposed.

KEY WORDS: Shell disease · Morphological characteristics · Fishing-induced injury · Melanisation · Total haemocyte count

INTRODUCTION

Commercial fisheries and aquaculture of marine lobsters globally are worth well in excess of US $2 billion yr^{-1} (Kough et al. 201). An increasingly common disorder in lobsters is a classical shell disease which encompasses a range of different conditions and etiologies that are externally apparent on the exoskeleton (Chistoserdov et al. 2005, Quinn et al. 2013a). Classical shell disease has been described in many lobster species, including the European lobster *Homarus gammarus*, the American lobster *H. americanus*, spiny lobsters (*Panulirus*, *P. cygnus* and *Jasus edwardsii*) and Norway lobster *Nephrops norvegicus* (Chistoserdov et al. 2005, 2012, Quinn et al. 2013a).

Among spiny lobsters, a commonly reported form of classical or endemic shell disease is tail fan necrosis (TFN), which is characterized by erosion and blackening of the biramous uropods (i.e. each comprising an exopod and an endopod) and telson that make up the tail fan (Musgrove et al. 2005, Mancuso et al. 2010, Shields 2011). This condition has been reported in a number of spiny lobster species from captive animals and wild populations (Ooi 2014). For

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example, between 33 and 43% of wild-caught adult Australasian red spiny lobsters *J. edwardsii* developed TFN after 30 wk of holding in sea-cages in south Australia (Bryars & Geddes 2005). Also, in north-eastern New Zealand, over 17% of adult male *J. edwardsii* sampled from a fished wild population over a 3 yr period of sampling were found to have TFN (Freeman & MacDiarmid 2009). By comparison, only 2% of lobsters within a nearby unfished marine reserve had TFN, which strongly indicated that handling by commercial fishers was a likely cause of the TFN in the lobsters living outside the reserve. This conclusion is supported by prior evidence that TFN is initiated by external physical damage to the exoskeleton, with subsequent invasion of the damaged tissues by opportunistic marine bacteria, such as *Vibrio* spp. (Geddes et al. 2004, Musgrove et al. 2005). Physical damage has also been implicated in the initiation of classical shell disease in the American lobster (Quinn et al. 2012). The reactions leading to wound repair in spiny lobsters include haemocyte aggregation and coagulation followed by melanisation of the wound area which produces the distinctive blackened appearance of the affected area of the exoskeleton (Theopold et al. 2004, Vafopoulou et al. 2007, Cerenius et al. 2008). Frequently, the blackened tissues associated with TFN extend beyond the site of the initial wound as further melanisation of tissues occurs as a result of an immune response which aims to isolate an advancing bacterial incursion in the infected tissues (Musgrove et al. 2005).

In addition to the physical barrier presented by the exoskeleton in spiny lobsters, the haemolymph and especially the haemocytes are thought to play the main role in pathogen defence in crustaceans (Evans et al. 2000, Destoumieux-Garzón et al. 2016). The wound repair processes are mainly mediated by haemocytes or antimicrobial peptides, and other haemolymph components (Roch 1999). The haemocytes are involved in many immune functions and wound repair, such as prophenoloxidase activation, deposition of melanin precursors and phagocytosis (Hernández-López et al. 2003, Theopold et al. 2004, Fotedar et al. 2006). As a consequence of the pivotal role haemocytes play in immune responses, total haemocyte counts and haemocyte viability are frequently used as measures of immune status in crustaceans. The recognition, inactivation and elimination of foreign organisms are effected through host defence responses involving circulating haemocytes, fixed phagocytes, agglutinins or lectins and antimicrobial factors present in the haemolymph of invertebrates (Cerenius et al. 2008, Vogt 2012). Immunorecognition is thought to be mediated in part through the prophenoloxidase system, which involves a cascade of serine proteases and prophenoloxidase present in the haemocytes which is activated by non-self molecules (Söderhäll & Sritunyalucksana 2002, Perdomo-Morales et al. 2007). Consequently, these various immune responses provide the basis of a range of measures that have been used for determining the immune status of crustaceans (Fotedar et al. 2001, 2006, Verghese et al. 2007, Hernroth et al. 2012). Despite the ready availability of these measures for determining the immune status of lobsters, they have not been used previously to determine whether the immune status of lobsters affected with TFN is impaired as a result of actively responding to an TFN infection. The limitations of the crustacean immune response to acute bacterial infection would suggest that TFN has the potential to lead to systemic infection of lobsters by pathogenic bacteria with the potential to ultimately overwhelm infected individuals (Ooi 2014). It is also possible that TFN develops after the host immunity has been compromised from the stress of handling and physical damage, a pathway which has been implicated in other types of classical shell disease (Tlusty et al. 2007). However, it is likely that a combination of both pathways contributes to the development of TFN in spiny lobsters.

In this study, we contrast the physical condition and immune status of lobsters affected with TFN with those of unaffected individuals to provide some insight into the aetiology of TFN in spiny lobsters. We applied commonly used measures of immune status to lobsters sampled from a commercially fished population of red Australasian spiny lobsters *J. edwardsii* in northeastern New Zealand characterized by relatively high prevalence of TFN (Freeman & MacDiarmid 2009). Total haemocyte count (THC), haemocyte viability, total protein concentration in plasma and haemocyte lysate supernatant (HLS), phenoloxidase (PO) activity in the HLS and haemolymph bacteria count by culture were used to assess the immune status of lobsters sampled from the fishery. Lobsters, both with and without TFN, were sampled and analysed for comparative purposes.

MATERIALS AND METHODS

Spiny lobster specimens

Wild adult and juvenile *Jasus edwardsii* were caught over 2 d in April 2016 using commercial lob-

ster pots on the east coast of the North Island of New Zealand (38° 37′ S, 178° 13′ E) in commercially fished areas previously identified to have high prevalence of TFN among lobsters (map included in Freeman & MacDiarmid 2009). A total of 44 lobsters with TFN and 38 without TFN were randomly selected from the entire catch and immediately placed in chilled insulated boxes and transferred to the laboratory within 2 h of capture for further processing. Other than initially categorising the captured lobsters into 2 groups (i.e. TFN present or absent) based on an initial examination of the lobsters immediately after capture, the lobster sample is representative of a typical lobster harvest from this location at this time.

Morphological measures and TFN assessment

The carapace length (CL) and tail width (TW) of all the lobsters were measured using calipers, and the number of missing appendages (antennae, pereiopods and pleopods) on individual lobsters was recorded. Moult stage of the lobsters was also recorded according to Musgrove (2000). For lobsters with TFN, the number of affected elements of the tail fan (i.e. exopods, endopods [uropods] and telson; see Fig. 2A) of each individual lobster was counted, including whether the ventral or dorsal parts of the tail fans were affected. The percentage of total areas of the ventral and dorsal surfaces of tail fans were separately estimated for each exopod, endopod and telson on each lobster that was affected by TFN. This estimate included an allowance for areas of each exopod, endopod or telson that was missing but for which the attached remaining margins were affected. The total area of the tail fan affected by TFN for each lobster was then calculated as the mean of the affected ventral and dorsal surface areas.

Haemolymph collection, THC and haemocyte viability assay

The bases of the fifth thoracic legs of all lobsters were disinfected with 70% alcohol, and a sample of haemolymph was removed with a 26 gauge needle attached to a 1 ml sterile syringe (Terumo). THC and haemocyte viability of all lobsters were assessed using a portable flow cytometer (Muse® Cell Analyser and Muse® Count & Viability Assay Kit; Merck Millipore). An aliquot of 100 µl of haemolymph was transferred to an equal amount of Alsever's solution (Sigma-Aldrich). Diluted haemolymph was then gently mixed twice to obtain a homogeneous suspension of haemocytes, and incubated at room temperature and then analysed on the instrument as per the manufacturer's instructions and as modified by Grandiosa et al. (2016).

Preparation of HLS

HLS was prepared from lobster haemolymph as per Perdomo-Morales et al. (2007) and Safari et al. (2015). Briefly, a 700 µl aliquot of haemolymph was mixed with anticoagulant (sodium citrate 0.114 M, sodium chloride 0.10 M, pH 7.45) before centrifugation at 800 × *g* (10 min at 4°C). The cell pellet was washed twice with anticoagulant, suspended in Tris-HCl (pH 7.5) and homogenized using a glass piston homogenizer. The lysate was then centrifuged at 10 000 × *g* (10 min at 4°C) to obtain the clarified HLS.

PO activity in HLS

The PO activity in the HLS of all lobsters was measured using previously described methods, with some modification (Hernández-López et al. 1996, Perdomo-Morales et al. 2007, Celi et al. 2015). An aliquot of 25 µl of HLS was incubated with 50 µl trypsin (1 mg ml^{-1}) in 50 mM Tris-HCl buffer (pH 7.5) for 20 min at room temperature in 96-well polystyrene plates. A control consisting of only buffer was also included. An aliquot of 50 µl L-DOPA (3 mg ml^{-1} in distilled water) was added to each well, and the absorbance was measured at 490 nm using a microplate reader after 10 min of incubation at 25°C. Enzyme activity was expressed as the change in absorbance at 490 nm min^{-1} mg^{-1} of protein in 10 min.

Total protein concentration in haemolymph plasma and HLS

The total protein concentrations in haemolymph plasma and HLS from all lobsters were determined using a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific) and Quant-iT™ Protein Assay Kit (Thermo Fisher Scientific) (Celi et al. 2015). Haemolymph was mixed with anticoagulant (sodium citrate 0.114 M, sodium chloride 0.10 M, pH 7.45). Plasma was extracted by centrifuging lobster haemolymph at 800 × *g*, and the supernatant was collected for the plasma protein assay. The HLS used for this assay was prepared in the manner described previously.

Haemolymph bacteria assessment

A 100 µl aliquot of haemolymph from each lobster and a 10-fold dilution (in autoclaved filtered seawater) were spread onto each of 3 replicate marine agar plates (BD Difco), and incubated at 18°C for 7 d. Colony-forming units (CFU) were counted and CFU ml^{-1} was calculated for each sample.

Statistical analyses

A *t*-test and Welch's *t*-test were used to compare the mean CL and TW of lobsters with and without TFN, respectively. Welch's *t*-test was used because of unequal variances between the 2 samples. A Pearson's chi-squared test was used to compare the proportions of male lobsters that were over the legal size limit (i.e. TW = 54 mm) for those individuals with and without TFN. This test was used because it determines whether proportions of categorical data were independent (Sokal & Rohlf 2012). A Pearson's chi-squared test was also used to compare the proportion of lobsters with and without missing appendages for the lobsters with TFN versus those lobsters without TFN. A Mann-Whitney test was used to compare the number of missing appendages between lobsters affected and not affected by TFN. The Mann-Whitney test was used because it provides a statistical comparison of 2 independent groups of discrete nonparametric variables (Sokal & Rohlf 2012). A Pearson's chi-square test was also used to compare the proportions of the different tail fan elements (i.e. exopod, endopod, telson) that were affected by TFN. The percentages of the ventral and dorsal surface areas of the tail fans of individual lobsters affected by TFN were compared with a paired *t*-test after the data were arcsine transformed to normalise their distributions (Sokal & Rohlf 2012).

Welch's *t*-tests were used to compare THC, haemocyte viability, PO activity, protein content in haemolymph plasma, protein content of HLS and haemolymph bacteria counts for lobsters with and without TFN, respectively. Percentages of haemocyte viability were arcsine transformed for analysis. Haemolymph bacteria counts were $\log_{10}$ transformed to improve homogeneity of variance, normality of the residual errors and additivity of treatment effects for analysis.

All results are presented as mean ± SE unless otherwise stated. Data analyses were undertaken using SPSS Statistics 23 software.

RESULTS

Morphological characteristics

The CL of the sampled lobsters ranged between 89 and 111 mm. Lobsters with TFN were significantly larger than those without TFN (mean CL 103 ± 0.6 mm versus 99.0 ± 0.7 mm; *t*-test, $t = -4.37$, $p < 0.001$). The TW of the sampled lobsters ranged from 43–57 mm. Lobsters with TFN had significantly larger TW than those lobsters without TFN (mean TW 52.6 ± 0.2 mm versus 51.4 ± 0.4 mm; Welch's *t*-test, $F = 6.26$, $p < 0.02$). The legal TW size is 54 mm for males, and 29.5% of male lobsters with TFN were legal size versus 18.4% for male lobsters without TFN ($\chi^2 = 1.37$, $p = 0.24$). The majority of the sampled lobsters were male, with a total of only 3 female lobsters sampled (all unaffected by TFN). All lobsters were at the inter-moult stage.

Lobsters with TFN were >4 times more likely to have a missing or injured appendage (i.e. antenna, pereiopod or pleopod) than lobsters without TFN (63.6 versus 18.4%; $\chi^2 = 17.04$, $p < 0.001$). Furthermore, lobsters with TFN had more missing or injured appendages than those without TFN (Mann-Whitney $U = -4.42$, $p < 0.001$; Fig. 1A). For example, among the lobsters with TFN, 1 lobster was missing a total of 8 appendages, whereas the maximum number of missing appendages for lobsters without TFN was only 3. Whilst sampling lobsters, we observed that lobsters with more advanced TFN generally appeared less vigorous in their responses to handling than those without TFN in terms of antennae waving, tail flicking, pereiopod grasping and strength of their pereiopod grip; however, these parameters were not quantified.

Of the 44 lobsters with TFN, 29.5% had TFN present in only 1 element of their tail fan, i.e. either an exopod, endopod or telson. A total of 29.5% of the lobsters had TFN present in 2 tail fan elements, 18.2% in 3 elements, 20.5% in 4 elements and 2.3% in all 5 elements (Fig. 1B). Necrosis had extended into the peduncle and the base of abdomen in 3 TFN-affected lobsters. In 1 lobster, necrosis was present in the anterior portion of the abdominal somites. Of the lobsters with TFN, the affected area of the tail fans affected with TFN of individual lobsters ranged between 3 and 54%. Eighteen percent of lobsters had less than 10% of their tail fan affected by TFN (Fig. 2A); 36% of lobsters had TFN affecting between 10 and 20% of the tail fan (Fig. 2B,C), whereas 46% lobsters had more than 20% of their tail fans affected

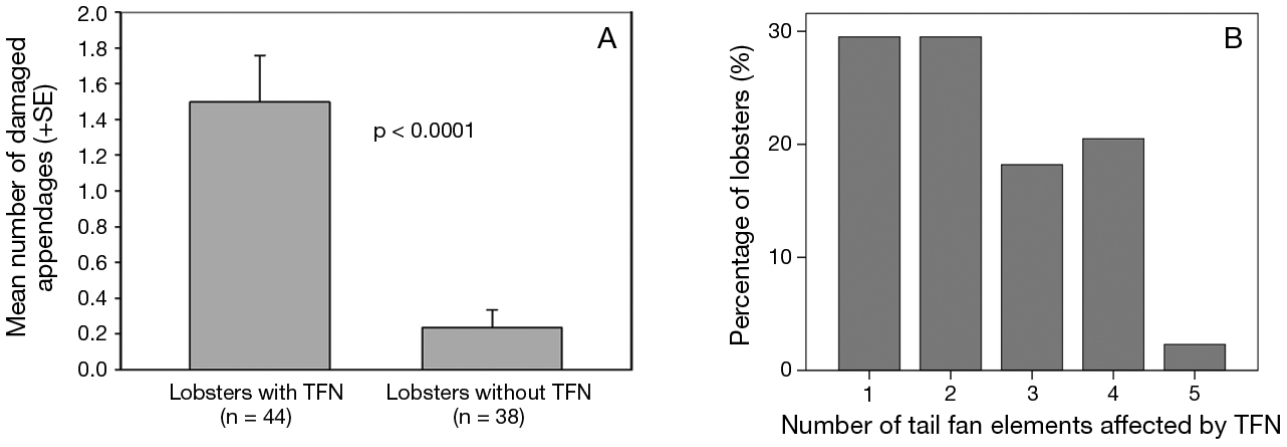

Fig. 1. Morphological characteristics of sampled spiny lobsters *Jasus edwardsii*. (A) Mean number (±SE) of damaged appendages (i.e. antennae, pereiopods and pleopods) in sampled lobsters with and without tail fan necrosis (TFN). (B) Number of individual elements of the tail fan (i.e. either an exopod, endopod or telson) affected by TFN in 44 lobsters

by TFN (Fig. 2D). TFN was more commonly found in endopods (59.1%) than in the exopods (43.2%; χ^2 = 4.5, $p < 0.04$) and in the telson (34.1%; $\chi^2 = 7.3$, $p < 0.01$). The mean surface area of the tail fan of lobsters that was affected by TFN was greater on the ventral surface than on the dorsal surface, i.e. 25.7 ± 2.1% versus 20.0 ± 1.9% (paired *t*-test, $t = 10.1$, $p < 0.0001$; Fig. 2B,C).

THC and haemocyte viability assay

The mean THC of lobsters without TFN was 3.01 ± 0.08 × 10^6 cells ml^{-1}, which was significantly higher than lobsters with TFN 2.77 ± 0.06 × 10^6 cells ml^{-1} (Welch's *t*-test, $F = 6.02$, $p < 0.02$). The mean haemocyte viability in lobsters without TFN was 89.4 ± 0.8%, which did not differ from lob-

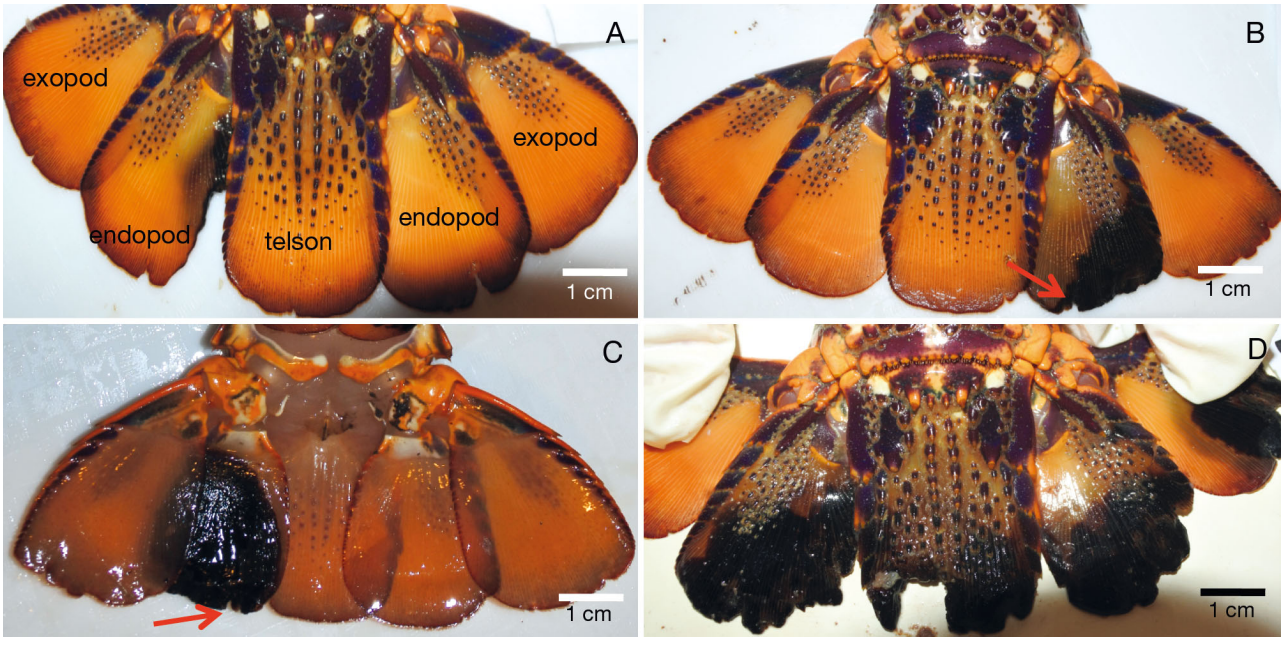

Fig. 2. Photographs of sampled spiny lobsters *Jasus edwardsii* showing typical appearance of tail fan affected with tail fan necrosis (TFN). (A) Lightly affected, <10% TFN, showing the 3 elements of the tail fan (dorsal surface). (B) Moderately affected, 10–20% TFN. Note the small notch (arrow) in the endopod from which the TFN appears to have radiated (dorsal surface). (C) Same tail fan as in (B), showing difference in extent of TFN between dorsal and moderately affected ventral surface (8 versus 18%, respectively). (D) Severely affected, >20% TFN (dorsal view)

sters with TFN 89.7 ± 0.3% (Welch's *t*-test, F = 0.04, p = 0.83).

PO activity in the HLS

The mean PO activity in the HLS of lobsters without TFN (1.11 ± 0.06 mg^{-1} min^{-1}) was significantly higher than that of lobsters with TFN (0.71 ± 0.05 mg^{-1} min^{-1}; Welch's *t*-test, F = 26.04, p < 0.001).

Total protein concentration in haemolymph plasma and HLS

The protein concentration of haemolymph plasma ranged from 22–95.6 mg ml^{-1}. There was no difference in the mean protein concentration of haemolymph plasma in lobsters without TFN (70.41 ± 2.48 mg ml^{-1}) versus lobsters with TFN (67.55 ± 2.90 mg ml^{-1}; Welch's *t*-test, F = 0.56, p > 0.45). The protein content in HLS ranged from 2–7.4 mg ml^{-1}. There was no difference in the mean protein concentration in HLS of lobsters without TFN versus those with TFN (4.40 ± 0.18 mg ml^{-1} and 4.40 ± 0.16 mg ml^{-1}; Welch's *t*-test, F = 0.01, p = 0.95).

Haemolymph bacteria assessment

Counts of haemolymph bacteria (>900 CFU ml^{-1}) were highly variable in both lobsters with and without TFN, with a small number of lobsters both with and without TFN having counts exceeding 900 CFU ml^{-1}. There was no significant difference in the mean haemolymph bacteria counts for lobsters without TFN (565 ± 267 CFU ml^{-1}) versus those with TFN (94 ± 34 CFU ml^{-1}; Welch's *t*-test, F = 0.51, p > 0.22).

DISCUSSION

This study is the first investigation of the physical condition, in terms of injury, and the corresponding immune status in a population of *Jasus edwardsii* with endemic TFN infections as evaluated by applying a range of immunological tools commonly used in crustaceans (Fotedar et al. 2006, Celi et al. 2015, Safari et al. 2015, Grandiosa et al. 2016).

The lobsters sampled from the population using commercial pot methods were dominated by males (96.3%) of a size close to the legal size limit of 54 mm TW, which is a characteristic of this coastal fishery where females seasonally migrate into deeper waters (Freeman & MacDiarmid 2009, Linnane et al. 2015). Male lobsters around legal size are the focus of commercial potting, with all captured lobsters handled for measuring, and if undersized are returned to the water (Starr et al. 2015). Sampled lobsters with TFN were larger in size (both CL and TW), which may be the result of TFN-affected animals being returned by commercial fishers who routinely sort their catch and only retain the highest value animals and return the remainder to the sea (known as 'high grading') in order to maximise the value from their catch quota (Sykes 2017). Three commercial fishers confirmed this behaviour because lobsters with TFN reportedly attract lower market prices due to their unsightly appearance and their reduced ability to withstand live transport to international markets. The effect of handling and returning diseased animals to the natural population may have important implications for the aetiology of TFN within the lobster population, especially for providing increased potential for cross infection whilst lobsters are corralled in pots, collectively handled by fishers, and whilst cohabitating shelters in the wild, as observed to be important for transfer of diseases in fished populations of other lobster species (Parsons & Eggleston 2005, Milligan et al. 2009, Leland et al. 2013, Campos et al. 2015, Emery et al. 2016). This is a possible explanation for the marked difference in the prevalence of TFN previously observed on the same coastline between an unfished lobster population in a marine reserve (<2%) versus the adjacent fished population (17%) (Freeman & MacDiarmid 2009).

Missing appendages were more commonly found in the TFN-affected lobsters, which is consistent with physical damage to the tail fan caused by capture in pots and post-capture handling initiating TFN (Musgrove et al. 2005, Milligan et al. 2009, Campos et al. 2015), and is also consistent with the findings of Freeman & MacDiarmid (2009) for lobsters in this area off the coast of New Zealand. In addition, TFN was also more prevalent on the ventral surfaces of tail fan elements than on the dorsal surface, which may be due to differences in thickness of the carapace on the ventral surface, or due to physical damage sustained while lobsters use the tail flip escape response.

For those lobsters with small areas affected by TFN, it was frequently possible to identify the extent of physical damage (i.e. loss and tears) to the tail fan from which the TFN appeared to have been initiated. In most cases, this consisted of a tear from the anterior margin of the tail fan element (i.e. telson, endopod or exopod) and extending into, and through, the

element. Less commonly, pieces of tail fan would have been removed in their entirety or there would be clear signs of a puncture wound. Collectively, these types of injury are characteristic of lobsters exposed to handling and holding in mesh enclosures (Musgrove et al. 2005). In contrast, in lobsters with more extensive TFN, it was frequently difficult to determine the point of initiation of TFN because of the extensive loss of tail fan tissue. This suggested that the condition had continued to advance well beyond the site of initial injury. The TFN-affected lobsters may be able to replace their affected tail fans during a moult, but previous studies indicate that increasing severity of TFN rather than recovery is more common in lobsters affected with TFN (Geddes et al. 2004, Musgrove et al. 2005). In the current study, there was no sign of recovery from previous TFN among the sampled lobsters, because moult scars are commonly observed in crabs and lobsters that have recovered from injuries (Deangelis et al. 2010).

Immune parameters of an animal may vary in response to different diseases or environmental challenges (Shields 2011). In the current study, of the 7 commonly used parameters to evaluate immune status in crustaceans, only 2 showed significant differences between lobsters with TFN and those without. The circulating haemocytes found in lobsters contribute to a series of primary immune actions, such as phagocytosis, coagulation, encapsulation and melanisation (Smith et al. 2003, Hernroth et al. 2004, 2012, Cerenius & Söderhäll 2012). THC was around 9% higher in lobsters without TFN versus those with TFN, while there was no difference in haemocyte viability. However, this would result in a higher relative number of viable haemocytes in the haemolymph of lobsters without TFN. This difference may be due to the loss of haemocytes in responding to the TFN infection. The lower abundance of haemocytes in the haemolymph of lobsters with chronic TFN has the potential to make them more prone to further invasion by the TFN infectives and any other invasive micro-organisms.

PO activity in the HLS was markedly higher (56%) in lobsters without TFN versus those with TFN in this study, which is likely to be the result of responding to the disease. PO activity is a key step of the prophenoloxidase activating system, which results in the production of highly reactive and toxic quinone intermediates and finally the melanisation of infected tissues (Cerenius et al. 2008, 2010). During this process, intruding protozoans and bacteria are frequently attacked and melanised in many invertebrates, including lobsters (Cerenius et al. 2010). Therefore, PO activity is an important measure of the primary immune response of a lobster to physical damage and microbial incursion arising from such damage. A lower PO activity in TFN-affected lobsters indicates that this PO response appears to be substantially compromised in lobsters with TFN, most likely as a result of active ongoing deployment against the chronic TFN condition resulting in the outward appearance of melanisation of the tissues.

Apparently healthy crustaceans, including lobsters, frequently have bacterial flora in their haemolymph (Fotedar et al. 2001, Chistoserdov et al. 2005, Meng et al. 2010, Wang 2011, Shields et al. 2012, Quinn et al. 2013b), although a study has shown that haemolymph in a small sample of lobsters was aseptic (Mancuso et al. 2010). Bacteria were also found in the haemolymph of healthy lobsters, which is consistent with previous studies. The overall bacteria counts in the haemolymph of healthy lobsters and those with TFN were similar, which suggests that bacteria count in haemolymph is not associated with TFN in lobsters. Bacteria counts from lobster haemolymph make no distinction between normal bacterial flora and potential pathogens. The limitation of this technique is further compounded by the inability of many bacteria to be cultured, which can result in underestimates in bacteria counts (Stewart 2012). Further application of molecular genetic methods would provide an opportunity to reliably identify the bacteria present in haemolymph that may not be culturable and that may also be associated with TFN.

There are a variety of proteins in haemolymph plasma and HLS, including haemocyanin protein, lectin, coagulogen, macroglobulin, subtilisin-inhibitor, trypsin inhibitor, clotting protein and PO (Söderhäll & Cerenius 1992, Theopold et al. 2004, Cerenius et al. 2008, Cerenius & Söderhäll 2012), which are all known to contribute to a series of immune actions in crustaceans, including lobster species (Söderhäll & Cerenius 1992, Fotedar et al. 2006, Cerenius et al. 2010, Clark 2014). Consequently, haemolymph protein concentration has been used as a proxy measure of health and immune status (Oliver et al. 2001, Fotedar et al. 2006, Verghese et al. 2007, Behringer et al. 2008, Shields 2011). For example, haemolymph protein concentration has been found to be significantly lower (by 4–5 mg l^{-1}) in Caribbean lobsters *Panulirus argus* infected with *P. argus* virus 1 (PaV1) compared with uninfected lobsters (Behringer et al. 2008). However, the lack of any apparent differences in protein levels in haemolymph plasma and HLS due to TFN infection suggests that the affected lob-

sters may not be greatly endangered by the presence of this disease as it is usually a marker for near-death condition in crustaceans.

It seems likely that physical damage to lobsters associated with fishing is an initiator of TFN in the population of lobsters in this study, as first suggested by Freeman & MacDiarmid (2009). If this is the case, then a number of measures can be taken to prevent and control the disorder. Commercial traps can be modified to have larger mesh sizes and softer surfaces, such as plastic-coated mesh, to minimise the physical damage to lobsters (Matthews 2001, Shields 2012, Leland et al. 2013, Butler & Matthews 2015). Likewise, less damaging handling systems can be set up on fishing vessels for landing and removing the catches from traps (Davidson & Hosking 2004). Handling gloves and commercial traps could be disinfected with weak chlorine bleach solution before use in fishing to decrease the possibility of cross contamination, as lobsters with TFN are likely to come into contact with both sets of surfaces, raising the potential for transfer of infective bacteria. Removal of the strict size limit might be another effective means for decreasing the damage to lobsters that is likely to result from repeated handling from intensively fishing to a size limit (DiNardo et al. 2002, Emery et al. 2016). Removal of diseased sublegal animals from the fishery could also decrease the chance of transmission among the lobster population (Shields 2012). Lobster fishing could also be curtailed during the peak lobster moulting period when exoskeletons are still hardening and more vulnerable to fishing-induced lacerations (Jeffs et al. 2013). Lobsters with TFN could be required to be landed rather than returned to the fishery in order to help prevent the possible persistence and transfer of the disorder through ongoing contact with other lobsters in traps and in natural shelters (Musgrove et al. 2005, Behringer et al. 2008, Shields 2012).

Only relatively recently has the significance of crustacean diseases emerged as a major concern to the global production of crustaceans (Evans et al. 2000, Diggles et al. 2002, Shields 2011, Behringer et al. 2012, Stentiford et al. 2012, Wu et al. 2014). For example, infections of PaV1 in the population of *P. argus*, which supports the largest spiny lobster fishery in the world, can cause high mortality (up to 60%) of juveniles (Behringer et al. 2011). PaV1-infected lobsters are also more vulnerable to co-infections, having almost 50% higher gill infestation by *Epystilis* sp. and *Zoothamnium* sp. compared to lobsters without PaV1 infection (Pascual Jiménez et al. 2012). Likewise, the prevalence of epizootic shell disease remains high (up to 40%) in American lobsters off the coast of southern New England, and the disease has played a major role in the decrease of the fished lobster stock in this area and even had a catastrophic effect on the American lobster fishery (Wahle et al. 2009, Castro & Somers 2012, Hoenig et al. 2017).

Injuries to lobsters are a common outcome of a variety of harvesting methods and are assumed to have relatively minor consequences to undersized animals returned to the wild, such as retarded growth whilst the lost appendages are replaced (Powrie & Tempero 2009, Frisch & Hobbs 2011, Leland et al. 2013, Emery et al. 2016). Furthermore, minor tissue damage, such as the small tail fan lacerations that appear to have led to TFN infection that were observed in the present study (Fig. 2B), have not typically been measured in previous studies or even considered to be of concern. However, if this damage leads to the establishment and persistence of disease in a lobster stock, then the ultimate consequences for the harvestable stock could be significantly greater than anticipated.

Acknowledgements. This work was supported by a scholarship from the China Scholarship Council to H.Z. Thanks to Jamie Quirk, Jieyun Wu and Kaiwen Yang for substantial help with field and lab work. We thank Jeffrey Shields for helpful input which greatly improved the final manuscript. This research was conducted under animal ethics approval no. 001471 from the University of Auckland operating under New Zealand's Animal Welfare Act 1999.

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Editorial responsibility: Jeffrey Shields, Gloucester Point, Virginia, USA

Submitted: January 18, 2017; Accepted: September 8, 2017
Proofs received from author(s): October 27, 2017