SCIENTIFIC PAPER

Fish Veterinary Journal (2012) (13) 18–30

Biofouling of the hydroid *Ectopleura larynx* on aquaculture nets in Ireland: Implications for finfish health

Emily J. Baxter[1,2,4*], Michael M. Sturt[2], Neil M. Ruane[3], Thomas K. Doyle[1], Rob McAllen[2] and Hamish D. Rodger[4]

[1]Coastal and Marine Research Centre, Environmental Research Institute, University College Cork, Naval Base, Haulbowline, Cork, Ireland
[2]School of Biological, Earth and Environmental Sciences, University College Cork, Distillery Fields, North Mall, Cork, Ireland
[3]Fish Health Unit, Marine Institute, Rinville, Oranmore, Co. Galway, Ireland
[4]Vet-Aqua International, Oranmore Business Park, Oranmore, Co. Galway, Ireland
*(E-mail: e.baxter@gmx.com)

Abstract

The potential direct health problems posed to marine-farmed salmonids by the biofouling hydroid Ectopleura larynx (Phylum Cnidaria, Class Hydrozoa) and in situ net washing processes to remove the fouling organisms have not yet been addressed. In an attempt to address the possible impacts, the rate of E. larynx growth on aquaculture nets over a net-cleaning cycle was assessed and Atlantic salmon (Salmo salar) smolts were exposed to hydroid-biofouled nets under experimental challenge. After only 1 week of immersion, there was a high settlement of E. larynx on net panels, with the maximum growth observed after 3 week of immersion. For the challenges trials, experimental treatment groups of S. salar were exposed to hydroid net panels or loose hydroid material for 11 hours under controlled conditions. Gills were examined for signs of gross damage and assigned a histopathological gill score. Prior to the experiment, the gills were healthy and did not show signs of damage from any insult. After exposure to E. larynx, focal areas of epithelial sloughing, necrosis and haemorrhage were visible on the gills under histopathology and a maximum gill score of 4 was observed. These results are the first in an investigation of this kind and suggest that E. larynx can damage the gills of S. salar. Further work on this area is vital to develop a better understanding of the pathogenesis of the damage caused by hydroids and their long-term effects on fish health, growth and survival.

Introduction

The accumulation of biofouling organisms on aquaculture nets poses considerable problems for the industry (Braithwaite & McEvoy 2004). Fouling organisms significantly increase the weight of cage and mooring systems and decrease water flow through the cages, which compromises the environmental quality for the fish (Cronin *et al.* 1999). The hydroid *Ectopleura larynx* (syn. *Tubularia larynx*, Phylum Cnidaria, Class Hydrozoa) is already one of the most common and problematic biofouling species for Norwegian finfish aquaculture (Guenther *et al.* 2009). This fast-growing species is also a prevalent fouling organism on Irish finfish farms (Fig. 1). Two net cleaning practices are common on marine finfish farms: (1) changing of fouled nets; and (2) *in situ* net washing using a high pressure jet washer (as described by Guenther *et al*. 2010). Previous studies have focussed on the settlement, development, attachment methods of biofouling hydroids on aquaculture nets, as well as the effects of anti-foulants. As yet there has been limited consideration of the potential direct impacts that biofouling hydroids could present to finfish health (Clark *et al.* 1997, Rodger 2007). Potential risks are that fish may suffer skin lesions from coming into contact with hydroid polyps fouling the net (due to the discharging of nematocysts (stinging cells)) and/or that the *in situ* net washing which blasts the hydroid polyps and their associated nematocysts into the water column, may cause gill pathologies if inhaled by the fish.. In an attempt to investigate the potential impacts of the hydroid *E. larynx* on finfish health, the present study was conducted with the aims of: 1) investigating the growth of *E. larynx* over a net washing cycle (typically every 3–4 weeks in summer) and quantifying the amount of material present at the point of net washing; and 2) determining the potential pathological damage of this growth of biofouling hydroids caused to the gills of Atlantic salmon smolts before and after simulated *in situ* net washing.

Materials and Methods

Hydroid quantification

The growth of *Ectopleura larynx* on aquaculture nets in Ireland was quantified over a net washing cycle (a 4 week period of growth in between the removal of fouling). Nylon net settlement panels (180 x 180 mm with 20 x 20 mm mesh aperture) attached to weighted PVC frames were deployed in triplicate array. From previous observations it was seen that hydroid biofouling covered the entire salmon cages from the surface to the bottom of the net

cone. Therefore, four triplicate panel arrays were deployed at a nominal depth of 5 m depth, from the pontoon of an Atlantic salmon cage, within a farm on the west coast of Ireland in August 2009. One triplicate array was removed every week after deployment for 4 weeks and immediately preserved in 4% seawater formalin. Any biofouling hydroids were identified to species level. The numbers of polyp heads m^{-2} were then counted and the stage of

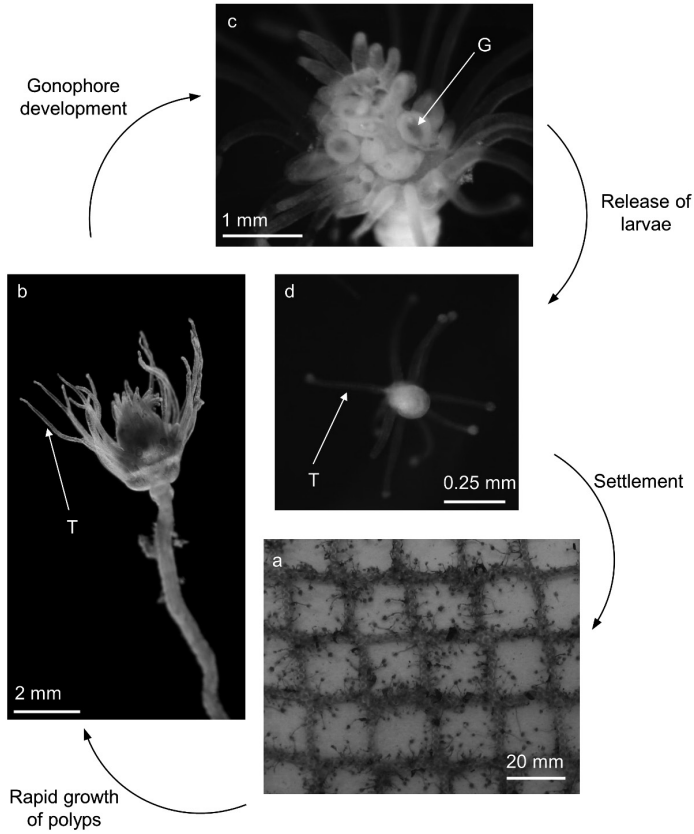

Fig. 1. Growth and development of the biofouling hydroid *Ectopleura larynx*. a) Settlement panel and growth after 1 week of immersion on a marine salmon farm; b) Developing polyp of *E. larynx* from settlement panels; c) close-up of polyp head showing gonophores; d) actinula larva after release from the gonophore. T = tentacle, G = gonophore.
Photo credits: a–b) E.J. Baxter; c–d) M.B. Belmar.

maturity of the polyps was assessed by the presence/absence of gonophores and actinula larvae within the gonophores (Fig. 1). The net panels were washed and then dried to a constant weight (overnight at 60°C). Desiccated colonies were brushed off the netting and weighed to obtain a dry weight of the fouling (g m^{-2}). The results providing the quantity of hydroid material present m^{-2} at the time of net washing and the likelihood of larval release. The difference in the number of polyps and dry weight between weeks was compared using one-way analysis of variance (ANOVA). Significant results were investigated *post-hoc* using Tukey's test.

Challenge trial

In a manner similar to the growth quantification, hydroids were cultivated *in situ* for the challenge trial during July 2010. Four large, nylon net panels (3 x 1 m in dimension) were deployed from a vacant cage pontoon. In addition, a small triplicate array (as described for the growth quantification) was deployed alongside for quantification of hydroid material used in the challenge trials. The panels were recovered 4 weeks later to coincide with the net changing regime on the farm. The panels were transported in cooled seawater to the Aquaculture and Fisheries Development Centre facility at the University College Cork, Ireland. Immediately prior to the challenge trials, the large net panels were fixed into a basket shape to be placed into the tanks. The experimental set-up consisted of one control and two experimental groups, each with duplicate tanks. For the control tanks, fish were exposed to completely clean (i.e. new) net baskets, whereas both experimental groups were exposed to hydroid colonised baskets. For the first experimental group, the hydroid colonised net baskets were placed directly into the tanks and for the second experimental group, the hydroids were scrubbed off each net panel and the scrubbed net baskets, plus the removed hydroid fragments were then added loose to the tanks to simulate the process of net cleaning. The outflows of all tanks were covered with 1mm stainless steel mesh to prevent large pieces of hydroid matter from leaving the experimental tanks and 5µm mesh mechanical filter bags were used in the sump filtration system to try and prevent hydroids or free nematocysts from entering the re-circulation system. The fish were exposed to the net baskets and/or hydroids for 11 hours before removal of all challenge material.

Hatchery-raised Atlantic salmon (*Salmo salar*) smolts (S1, 1 year old) were obtained from the Marine Institute hatchery, Furnace, Newport, County Mayo, Ireland and seawater adapted on arrival at the Aquaculture and

Fisheries Development Centre, University College Cork, Ireland. At the start of the challenge there were 30 fish in each group with an average weight of 119.1 ± 28.4 g (mean ± 1 S.D., n = 65) and were kept at a stocking density of 4.0 kg m^{-3}. All tanks contained 300 L of flowing seawater (salinity: 33 practical salinity units (psu)) at 11 ± 1°C with a supplementary air supply to keep dissolved oxygen levels >7.5 mg L^{-1}. The fish were maintained under a 12:12 hour light to dark photoperiod and fed a commercial pellet diet (Skretting: Atlantic smolt) throughout the day by automatic feeders.

Fish were kept off feed for 24 hours prior to the start of the challenge. At time 0 hours (prior to challenge) 5 fish were randomly sampled from the entire population. Five fish were then sampled from each tank at 2, 6, 24, 48, 168 (1 week) and 504 (3 week) h from the start of the experiment. Sampling was performed *post-mortem* and fish were euthanized with a lethal dose of the anaesthetic Tricaine methane sulfonate (MS-222, Pharmaq). The skin and gills of the fish were first examined for gross pathological changes; the second gill arch on the left-hand side was excised from each fish and immediately fixed in 10% neutral-buffered formalin for histology. The gills were paraffin embedded, cut into 5µm sections and stained with haematoxylin and eosin. All slides were scanned microscopically at increasing magnifications (40x, 100x and 400x). On histopathological examination, the gills were inspected for signs of gill damage (i.e. epithelial hyperplasia, necrosis, oedema, inflammation) and scored using the semi-quantitative gill scoring methodology, developed by Mitchell *et al.* (2012) and the clinical significance of any such damage was suggested. One-way ANOVA was used to test for a difference in the gill scores between test and control groups.

Results

Hydroid growth

During the initial hydroid quantification aspect to the study in August 2009, there was a high coverage of a single hydroid species, *Ectopleura larynx*, on the settlement panels from 1 week (mean ± SE: 54,290 ± 1759 polyp heads m^{-2}; dry weight 8 ± 0.04 g m^{-2}) onwards (Fig. 2). During the following 2 weeks there was a significant increase in the number of polyps and dry weight to the maximum recorded at 3 weeks (mean ± SE: 88,683 ± 4520 polyp heads m^{-2}; dry weight 56 ± 1.0 g m^{-2}) (Fig. 2a–b). At 4 weeks of immersion the number of polyp heads and the dry weight recorded decreased (Fig. 2a–b) as autotomy (the loss of the polyp heads before regeneration) of

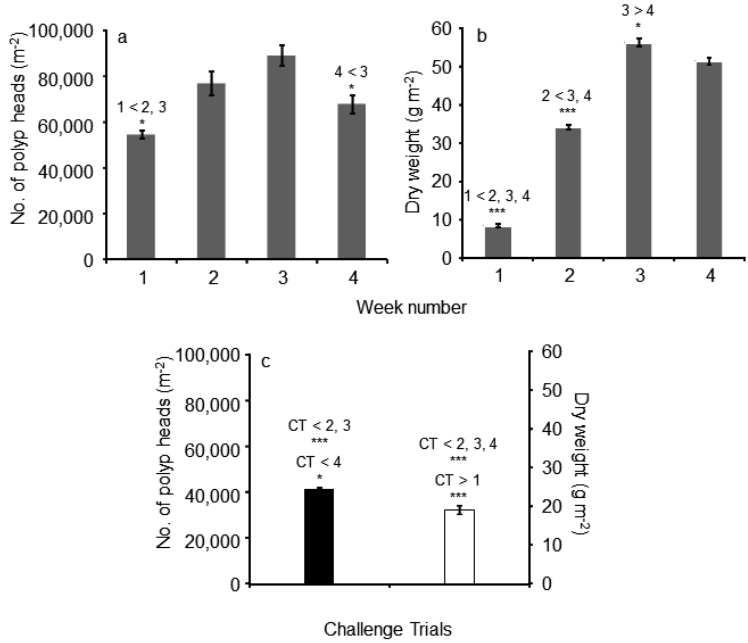

Fig. 2. Quantification of the hydroid *Ectopleura larynx* fouling aquaculture nets in Ireland. a–b) By week from immersion in the preliminary quantification over a net changing cycle; and c) as used for challenge material in the experimental trial. All values are means ± SE. Significance levels from pairwise comparisons: * $p < 0.05$, *** $p < 0.0001$

most of the polyps had occurred. The development of gonophore buds was apparent on some polyps from 1 week, and at 2–3 weeks actinulae (larvae) were visible inside the gonophores, some of which remained until 4 weeks.

Challenge trial

In the quantification of the challenge material in July 2010, there was also a high coverage of *Ectopleura larynx* on the large panels and the triplicate array after 4 weeks of immersion. However, many of the polyp heads had already been autotomised at this point. Therefore, although the dry weight of hydroid material on the panels was significantly higher than the Week 1 panels of the original quantification study, the number of polyp heads was significantly lower than Weeks 2, 3 and 4 (Fig. 2a–c).

In response to exposure to net baskets biofouled by *Ectopleura larynx* or loose hydroids, gross pathological changes of the gills were observed in fish from the experimental treatment groups. Focal haemorrhages were visible on the gills of some fish after 2 hours of exposure and a fragment of hydroid was found within the gills and gill rakers of one fish (see Fig. 3). Clouding of the cornea was also observed possibly from abrasion on the net. No other lesions were visible grossly on external or internal examination of the fish at any stage. Under histopathological examination, it was clear that all of the gills sampled at time 0 hours had good health and there were no signs of pathological gill damage. However after the commencement of the experiment, the gills of all groups (control and experimental) were observed with focal areas of epithelial sloughing, necrosis and haemorrhage (Fig. 4b–d). The gill histopathology observed was variable over the course of the trial in both severity and between groups. The range of the gill scores of both the control and treatment groups was between 0–4 throughout the trial (Fig. 5). There was no significant difference in gill scores between the different groups. The gill damage observed on all occasions was considered acute but not severe. In marine-farmed salmon this degree of damage has been associated with lethargy and a decrease in appetite, but as yet, not mortalities (Rodger *et al.* 2011b).

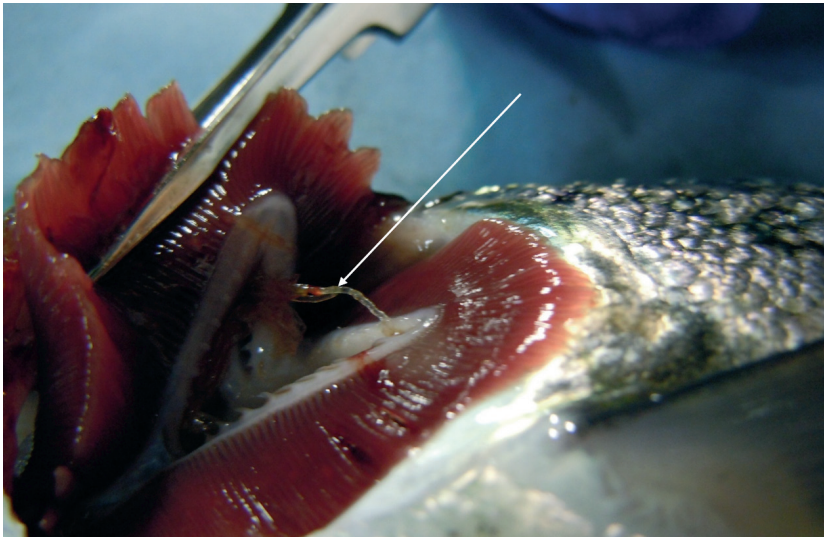

Fig. 3. Fragment of *Ectopleura larynx* (indicated by arrow) observed under gross pathology in the gills of an Atlantic salmon smolt during the challenge trial (experimental group: biofouled net – 2 hours from start of challenge).

Discussion

Several jellyfish species have been implicitly linked to fish kill events (Båmstedt *et al.* 1998, Purcell *et al.* 2007) and fish health issues, including gill damage (Baxter *et al.* 2011a, Baxter *et al.* 2011b, Rodger *et al.* 2011a), in marine-farmed salmon over recent years. Nevertheless, investigations into the potential effects of cage-fouling hydroids have been overlooked until now. The present study is the first to explore the direct effects of biofouling hydroids on the health of marine-farmed finfish; as opposed to the indirect effects, such as reduced flow (Cronin *et al.* 1999).

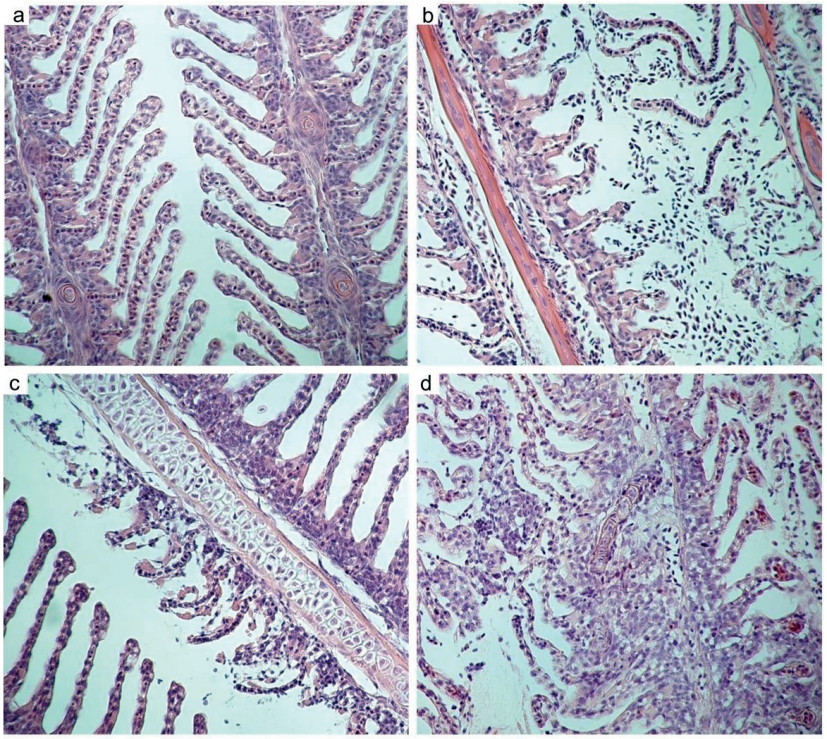

Fig. 4. Histological gill sections of Atlantic salmon smolts (*Salmo salar*) from control and experimental treatment groups used in the hydroid challenge trial. a–d) Gills from the different groups with time from the start of the experiment: a) 0 hours; (b) control group at 6 hours; (c) treatment group with biofouled net baskets at 48 hours; and (d) treatment group with cleaned baskets and free hydroids at 48 hours. Using haematoxylin and eosin (200x magnification).

This study documents that the biofouling hydroids have the potential to damage marine-farmed salmon in two ways: 1) the rapid growth of hydroids results in a dense growth of hydroid polyps bearing nematocysts which present a harmful surface to salmon – contact with hydroid polyps can cause gill (see Fig. 3) and potentially skin lesions; 2) the removal of the hydroids from the cages by *in situ* net washing can cause gill damage by suspending hydroid fragments and loose nematocysts. Here, no gill pathologies were observed prior to the challenge. However, after the start of the challenge acute gill pathologies were noted after exposure to hydroids biofouling the nets (Figs. 3 & 4) and loose hydroids after simulated net washing (Fig. 4). The gill scores observed across all fish throughout the challenge trial reached a maximum score of 4 (Fig. 5), rated as gill pathology of minor clinical significance (Mitchell *et al.* 2012). Although pathologies were observed in all groups, there were no other causative agents of gill disorders within the re-circulation system. No bacteria, amoebae, parasites or adverse environmental conditions were found, indicating that the damage observed was not caused by anything other than physical or toxic damage from the hydroid nematocysts. Thus, even though the control groups showed signs of gill damage this was likely because the tiny fragments of hydroids or nematocysts entered the re-circulation system. The gill damage observed was considered acute and may have caused lethargy and decreased appetite in the fish farm environment (Rodger *et al.* 2011b).

Recently, concerns about the potential problems caused by *in-situ* net washing have been raised by fish farmers throughout Ireland. There have been reports from divers working on site during net cleaning that there is an evasive response by the fish (moving away from the material removed by the jet washers). The dense clouds of debris that are blasted off the nets can be picked up on the boat echo sounders (S.O. Mitchell, pers. comm.) and although, cleaning is aimed to be conducted down-tide of the cages, it is nearly impossible to leave adjacent cages unaffected when there are many cages on-site.

The present study identified just how dense these clouds of biofouling material can be with up to 88,683 ± 4520 polyp heads m^{-2} (mean ± SE) of cage netting being blasted into the water. There is both anecdotal and experimental evidence to suggest that net washing practices can increase the grow-back rate of biofouling hydroids and cause the gonophores to burst and release larvae, which are then free to settle on the available space of the cleaned netting (Carl *et al.* 2010). The results presented here show that the potential forced

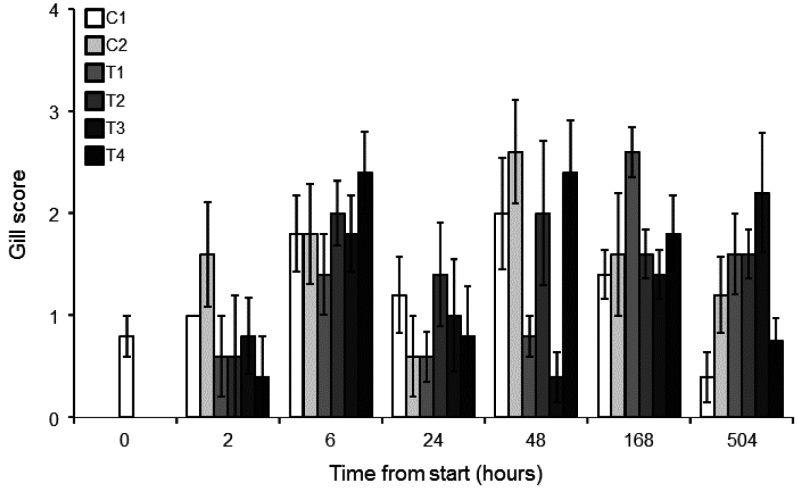

Fig. 5. Histopathological gill scores (± SE) of Atlantic salmon smolts (*Salmo salar*) sampled from the control and experimental treatment groups over the course of the hydroid challenge. C1 and C2: control groups; T1 and T2: treatment groups with biofouled net baskets; T3 and T4: treatment groups with cleaned baskets and free hydroids. All values are means ± SE (n = 5).

release of the larvae could occur in biofouling hydroid polyps only 2 weeks old (larvae were visible inside the gonophores at this stage), if removed by *in situ* net washing. Therefore, due to the rapid growth of this species, it is unlikely that cleaning the nets after 2 or 3 weeks would much reduce the number of polyp heads and/or larvae blasted into the water column (see Fig. 2) or reduce the grow back rate of the hydroids. *In situ* net washing practices are now becoming more common due to the quicker and less labour intensive nature of this method compared to traditional net changing methods. Given this move, it is imperative that future studies further investigate the potential implications of hydroid removal on fish health.

Acknowledgements

This work was part of the GILPAT project (Grant-Aid Agreement No PBA/AF/08/002(01)) carried out under the Sea Change strategy with the support of the Marine Institute and the Marine Research Sub-Programme of the National Development Plan 2007–2013 and co-financed under the European Regional Development Fund. EJB, TKD, and HDR were supported by the

above grant aid. All procedures were approved by the University College Cork Animal Experimentation Ethics Committee (review 2009/#41) and conducted under the Cruelty to Animals Act, 1876 (as amended by European Communities Regulations 2002 and 2005), licence number B100/4280. The authors would like to acknowledge the technical staff at the School of Biological, Earth and Environmental Sciences, the management of the Aquaculture and Fisheries Development Centre and Biological Services Unit (all University College Cork) for facilitating and supporting this research. Thanks also to D. Cotter for raising and supplying the Atlantic salmon smolts, C. Hickey for processing the tissues for histology, S.O. Mitchell and the salmon farm where the hydroids were cultured for their assistance in the experiments and M.B. Belmar for the use of her photographs.

References

Båmstedt, U., Fosså, J.H., Martinussen, M.B. & Fossahagen, A. (1998) Mass occurence of the physonect siphonophore *Apolemia uvaria* (Lesueur) in Norwegian waters. *Sarsia,* **83**, 79–85.

Baxter, E.J., Rodger, H.D., McAllen, R. & Doyle, T.K. (2011a) Gill disorders in marine-farmed salmon: investigating the role of hydrozoan jellyfish. *Aquaculture Environment Interactions,* **1**, 245–257.

Baxter, E.J., Sturt, M.M., Ruane, N.M., Doyle, T.K., McAllen, R., Harman, L. & Rodger, H.D. (2011b) Gill damage to Atlantic salmon (*Salmo salar*) caused by the common jellyfish (*Aurelia aurita*) under experimental challenge. *PLoS One,* **6**, e18529

Braithwaite, R.A. & McEvoy, L.A. (2004) Marine biofouling on fish farms and its remediation. *Advances in Marine Biology,* **47**, 215–252.

Carl, C., Guenther, J. & Sunde, L.M. (2010) Larval release and attachment modes of the hydroid *Ectopleura larynx* on aquaculture nets in Norway. *Aquaculture Research,* **42**, 1056–1060.

Clark, A., Nowak, B., Handlinger, J., Munday, B.L. & Percival, S. (1997) Clubbing and necrosis gill (CNG) syndrome in sea-caged Atlantic salmon, *Salmo salar* L., in Tasmania: an initial report. *Journal of Fish Diseases,* **20**, 59–68.

Cronin, E.R., Cheshire, A.C., Clarke, S.M. & Melville, A.J. (1999) An investigation into the composition, biomass and oxygen budget of the fouling community on a tuna aquaculture farm. *Biofouling* **13**, 279–299.

Guenther, J., Carl, C. & Sunde, L.M. (2009) The effects of colour and copper on the settlement of the hydroid *Ectopleura larynx* on aquaculture nets in Norway. *Aquaculture,* **292**, 252–255.

Guenther, J., Misimi, E. & Sunde, L.M. (2010) The development of biofouling, particularly the hydroid *Ectopleura larynx*, on commercial salmon cage nets in Mid-Norway. *Aquaculture,* **300**, 120–127.

Mitchell, S., Baxter, E.J., Holland, C. & Rodger H.D. (2012) Development of a novel histopathological gill scoring protocol for assessment of gill health during a longitudinal study in marine-farmed Atlantic salmon (*Salmo salar*). *Aquaculture International*, DOI: 10.1007/s10499-012-9504-x, Online First: February

Purcell, J.E., Uye, S. & Lo, W.-t. (2007) Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. *Marine Ecology Progress Series,* **350**, 153–174.

Rodger, H.D. (2007) Gill disorders: an emerging problem for farmed Atlantic salmon (*Salmo salar*) in the marine environment? *Fish Veterinary Journal,* **9**, 38–48.

Rodger, H.D., Henry, L. & Mitchell, S.O. (2011a) Non-infectious gill disorders of marine salmonid fish. *Reviews in Fish Biology and Fisheries,* **21**, 423–440.

Rodger, H.D., Murphy, K., Mitchell, S.O. & Henry, L. (2011b) Gill disease in marine farmed Atlantic salmon at four farms in Ireland. *Veterinary Record,* **168**, 668.

Emily Baxter graduated from a BSc (Hons) in Marine Biology and Oceanography at University of Plymouth in 2008. She then completed a PhD on "Gelatinous zooplankton in Irish waters: ecology and role in the gill disorders in marine-farmed salmon" at the University College Cork, Ireland in 2011. She is continuing her research on jellyfish and their detrimental impacts on aquaculture, as well as providing a zooplankton monitoring service as part of Vet-Aqua International.

Mike Sturt graduated from a BSc (Hons) in Biology at the University of Leeds and an MSc in Ecology from the University of Wales, Bangor. In 2010, he was awarded a PhD in freshwater ecology from University College Cork. His research focussed on nutrient limitation of food webs and top-down control of algae in freshwater ecosystems. Mike now works on river restoration projects in the UK.

Neil Ruane was awarded a BSc (Hons) in Zoology (1994) and an MSc in Aquaculture (1996) from University College Cork. In 2002, he completed a PhD at Wageningen University and Research Centre, The Netherlands and has since conducted research at the University of Algarve and University College Cork. His research interests include health and welfare of farmed fish. In 2005 he joined the Marine Institute as the Finfish Health Team Leader and is currently involved in several collaborative projects investigating diseases in Irish farmed fish.

Rob McAllen is a marine biologist with a number of different research areas, including conservation, sustainability and animal adaptations to natural and anthropogenic disturbances. He has been a lecturer within the School of Biological, Earth and Environmental Sciences, University College Cork since 2002 and research co-ordinator for the laboratories at the Lough Hyne Marine Nature Reserve in West Cork, with previous positions held at University of Aberdeen and University of York.

Thomas Doyle is a post-doctoral researcher at the Coastal & Marine Research Centre, University College Cork. He has been investigating the ecology and socio-economic impacts of jellyfish in Irish and UK waters under national and European research grants.

Hamish Rodger graduated from the University of Glasgow Veterinary School in 1984. He was awarded an MSc in Aquatic Veterinary Studies and a PhD from the Institute of Aquaculture, University of Stirling, Scotland. He is founder and principal veterinarian of the veterinary aquatic animal practice, Vet-Aqua International, and now practises aquatic animal veterinary medicine and conducts research into the health challenges of farmed finfish.

This paper was submitted on 17th February 2012 and accepted for publication on 20th June 2012.