Hydrozoan jellyfish blooms and interactions with Scottish salmon aquaculture: prediction, prevention and mitigation

by

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*Phialella quadrata* medusa (Hydrozoa, Leptomedusae.)
Photo by Otto Larink, courtesy of Scottish Association for Marine Sciences.
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I. Introduction

*Phialella quadrata* (Subclass Leptomedusae, Order Leptotheclata, Family Phialellidae) is a colonial hydrozoan species endemic to the British isles, with widespread distribution including inshore waters off northern Europe, Morocco, Japan, New Zealand, and Chile (Russell 1953, Cornelius 1955). In Scotland, it has produced two recorded large-scale blooms at salmon aquaculture facilities, in 1984 and 2008 (Bruno & Ellis 1985, Ferguson et al. 2010). Both blooms resulted in up to 90% mortality in caged fish, recently forcing the closure of a farm (Ferguson et al. 2010). A subsequent study showed that the medusae were causing damage to the fish through a combination of envenoming and transmission of bacterial disease, marking a first known instance of this phenomenon occurring in pelagic cnidarians (Ferguson et al. 2010). The present state of knowledge of hydrozoan blooms precludes informed prediction of blooms such as these, leaving aquaculture facilities open to similar incidents.

The goals of this review will be to examine the physical and biological interactions which may have an effect on the appearance of *P. quadrata* medusae in the plankton, and to consider its predatory ecology and symbiosis with *T. maritimum*. This information will be used to develop data-gathering procedures aimed at developing sustainable ways of mitigating the species’ potential effect on the aquaculture industry, and contribute to the field of zooplankton seasonal ecology and an emerging pool of research into cnidarian-prokaryote symbiosis.

II. Jellyfish and Scottish industry: current state of knowledge and future outlook

Impacts of jellyfish blooms and individual medusae on various coastal industries have been covered previously in numerous review papers (e.g. Seaton 1989, J. Purcell et al. 2007, Richardson et al. 2009, Nickell et al. 2010), but nonetheless bear brief reiteration. In Scotland, marine industries are vulnerable to cnidarian blooms by way of stock losses in aquaculture, interference with coastal power generation, and competitive or predatory interactions with commercial finfish species; while the latter two are worth considering, the main focus of my research will be toward the ecological factors affecting Scottish salmon production.

The British Isles are host to two classes of cnidarian that produce pelagic medusae. The Scyphozoa include 13 endemic species, which tend to be larger and more easily identified than the Hydrozoa, which include about 90 species (Russell 1953, Nickell et al. 2010). Members of both classes have been involved in fish kills at aquaculture facilities. The first reported damage to aquaculture took place in 1984, with a *Phialella quadrata* bloom that caused mass mortality of salmon smolts in Shetland in 1984 (Bruno & Ellis 1985). Since then, various species of scyphozoan and hydrozoan medusae including *Cyanea capillata* and *Aurelia aurita* (Seaton 1989, Baxter et al. 2011, Mitchell & Rodger 2011), *Solmaris corona* (Tørud & Håstein 2008), and *Pelagia noctiluca* (Doyle et al. 2008) have also been implicated, and other jellyfish-related fish kills have been reported without identification of the species involved (McKibben & Hay 2002). Mechanisms for mortality may include direct results of jellyfish envenoming via stinging, clogging of net pens resulting in localized anoxia, and spreading of disease (Doyle et al. 2008, Tørud & Håstein 2008, Mitchell et al. 2011). Outside of Scotland, but near enough to be of concern, the same list of cnidarian species plus others such as *Apolemia uvaria* and *Muggaiea atlantica* have caused mortality of
caged salmon in Norway and Ireland (Tørud & Håstein 2008). It has been suggested that worldwide jellyfish populations, and associated deleterious events, may be on the rise (Purcell et al. 2007, Richardson et al. 2009). Shifts toward cnidarian-dominated food webs, decreased finfish landings, and numerous blooms which interfere with power stations, have received increasing attention in the last decade (Lynam et al. 2005, Purcell et al. 2007, Richardson et al. 2009, Miller 2011). An increasing body of evidence suggests that larger and more frequent jellyfish blooms are to be expected in the future, due to factors such as climate change and overfishing (Richardson et al. 2009). It is reasonable to suspect that P. quadrata and other British cnidarian species will follow suit.

As stated previously, Phialella quadrata blooms have been implicated in two major incidents of salmon mortality in Scottish aquaculture. Both blooms occurred in Shetland, though concerns regarding the presence of the medusae throughout the salmon-producing areas of Scotland are mentioned in various reviews (e.g. Seaton 1989, Nickell et al. 2010). In the 2008 bloom, 90% loss of salmon stock took place over the course of about eight weeks, with fish eventually dying of respiratory failure as a result of severe gill lesions (Bruno & Ellis 1985, Ferguson et al. 2010). Jellyfish were present for most of this time, though their first appearance was not marked; it is approximated that they were present for at least six of the eight weeks in question. It was found that the P. quadrata medusae appeared to be transmitting the infectious bacterial species Tenacibaculum maritimum, probably accounting for at least some of the damage to gill tissue (Ferguson et al. 2010). The causative strain of T. maritimum was matched to a strain found living on the mouth parts of the medusae (Ferguson et al. 2010), raising a number of pertinent questions regarding the aetiology of infection and the symbiosis between P. quadrata and T. maritimum. These questions will be further considered in Part VI.

III. Life cycle of Phialella quadrata

In examining the ecological context of hydromedusan blooms, it is important to understand the life cycle features that contribute to bloom appearance. Hydrozoans undergo an alternation between a colonial benthic stage and a freeswimming medusa stage. Gametes from pelagic medusae are externally fertilised and zygotes develop into a motile planula larva, which eventually settles onto a substrate; initial reproduction takes place via asexual budding into a colony of polyps connected by hydrorhizae (Boero 1987). These are specialized either as feeding hydranths, or as medusa-producing gonangia. Colonies are dioecious and release only female or male medusae. Once in the water column, medusae mature and release gametes. The timing of this life cycle varies across taxa, but tentative estimates of time to maturity in P. quadrata are approximately 15-30 days (based on P. fragilis, a former conspecific) (Boero, 1987). Total lifespan in the plankton may range up to 70 days, though medusae typically begin to deteriorate once gamete release has occurred (Boero 1987).
Figure 1. Representative life cycle of *Phialella fragilis* (formerly conspecific with *P. quadrata*). Dioecious hydroid colonies consist of interconnected sessile polyps, of two distinct types: reproductive gonangia and feeding hydranths. Gonangia (a) produce the pelagic, sexually reproducing medusae, which develop within the protective gonotheca (b) before being released into the plankton. Colonies grow asexually, forming both lateral creeping hydrorhizae (c) and branching stalks (d). Hydranth polyps are specialized for feeding only, and do not produce medusae (e: retracted hydranths; f: extended, actively feeding hydranth). After release from the gonangia, juvenile medusae emerge with 4 tentacles at about 0.6mm in bell diameter (g). After 5-7 days, the medusae will reach about 2mm bell diameter with 8 tentacles (h). After about 12 days, medusae become fully mature, with a maximum bell size of 8-10mm in *P. fragilis* and up to 16mm reported in *P. quadrata* (i), and up to 16 tentacles. Gametes are released and fertilized externally, usually between 12-30 days after medusa release (j). Zygotes (k) develop in the plankton into ciliated planula larvae (l). After this several hours to several days, planulae attach by the anterior pole to an appropriate substrate and cells collapse around this point, eventually re-developing into the tissues of the primary polyp of a new colony (m), which increases in size by asexual budding (n). Drawn by A. Kintner, from Boero (1987).

Both the benthic and pelagic phases are important to understand in predicting medusa blooms. While it is the medusa life stage that causes damage to industry, it is the colonial hydroid stage that gives rise to medusae, necessitating an investigation of its reproductive dynamics. First, the proportion of feeding hydranths to reproductive gonangia is important: new polyps in a colony will become specialized as one or the other, but not both. A useful line of inquiry would be the factors influencing colonial growth and
the means by which polyps specialize, providing information toward estimating the potential magnitude of a bloom produced by a hydroid colony. Second, *P. quadrata* blooms are evidently seasonal, with the greatest number of medusae occurring during the summer months (Russell 1953, Bruno & Ellis 1985, Ferguson et al. 2010), but there is a paucity of data supporting speculation as to what physical or biological triggers lead to medusa production and release by the hydroid colony. An accurate forecast of medusa appearance based on environmental conditions would provide the obvious benefit of enabling aquaculturists to make spatial and temporal adjustments to their production timelines in order to avoid blooms. Third, once released, the medusae remain in the plankton for a mean duration and may even overlap with newly released generations of medusae; an accurate estimation of the lifespan in the plankton would be useful in gauging the length of time a bloom could pose a threat to industry. Procedures for gathering and analyzing data toward these ends are laid out in Part IX.

**IV. When and why do hydrozoan blooms occur?**

As previously described, the appearance and magnitude of *Phialella quadrata* blooms will be dependent on hydroid colonial capacity to produce medusae (i.e. number of gonangia) and a stimulus or stimuli to actually produce them. A wide variety of physical and biological conditions conducive to medusa production and colony growth are cited for other hydrozoan species, including light (Brinckmann-Voss 1985, regarding *Sarsia princeps*; Costello 1988, regarding *Cladonema californicum*), temperature (Werner 1958, regarding *Rathkea octopunctata*, and Werner 1961 and 1962 regarding *Bougainvillea superciliaris*, in Arai 1992; Widmer 2004, regarding *Mitrocoma cellularia*), salinity (Goy 1973, regarding *Scolionema suvaensis*), food availability (Roosen-Runge 1970, regarding *Cltyia gregarium*; Miglietta et al. 2008 regarding numerous hydrozoan taxa), lunar phases (Elmhirst 1925, regarding *Obelia geniculata*; Goy 1973, regarding *Scolionema suvaensis*), and various interactions amongst the above (Edwards 1978 & 1983, regarding *Sarsia occulta*, *S. tubulosa*, and *S. cliffordi*; Ma & Purcell 2005, regarding *Moerisia lyonsi*). *M. lyonsi* in particular showed increased gonangium ratios amongst the colonial polyps and a resulting increase of medusa release in response to rising temperatures, by as much as 25% per 1° C rise in temperature (Purcell 2005). Alternatively, some species release medusae with regularity without apparent regard to particular physical stimuli (Kubota 2008, regarding *Eugymnanthea* sp.). In some cases, these conditions are met as a constant; that is, a particular species’ colony held at 11° Celsius will produce medusae regularly and continuously and will do so significantly less at over 15° Celsius (Widmer 2004, regarding *Mitrocoma cellularia*); other conditions, such as a peak in prey abundance, may lead to similarly temporary peaks in medusa production (e.g. Arai 1987, regarding *Sarsia cliffordi*). This may represent an ecological situation in which resources are sufficiently flush that the hydroid colony can invest resources in sexual reproduction. In other cases, the stimulus for medusa production may be hormetic; that is to say, a short-term stressor such as rapidly dropping salinity, a damaging rise in temperature, or chemical toxicity appearing in the water column may cause a benthic colony to produce motile medusae which can escape unfavourable conditions, as well as increase diversity and population fitness through genetic recombination (e.g. Stebbing 1981, Stebbing 2002, Widmer 2004).

Many of these conditions will be environmentally dependent, but the bulk of published works regarding wild medusa populations in response to environmental flux has focused on larger scyphozoan medusae.
rather than the Hydrozoa. However, scyphozoan species studied have shown marked correlation between climatological and oceanographic effects. For example, the Scyphozoan species including *Cyanea capillata*, *C. lamarckii*, and *Aurelia aurita* have been found to have medusa populations correlating with the North Atlantic Oscillation, with positive NAO phases leading to cooler water in the North Sea and greater numbers of medusae (Lynam et al. 2004). Work is now underway to further define these effects on scyphozoan polyp strobilation and metamorphosis rates in laboratory cultures of these species (Widmer 2011). Furthermore, a number of coastal scyphozoan species have shown increased medusa population in response to eutrophication, in particular from nitrogen and phosphorus runoff, which tends to favour nutrient pathways toward low-energy species such as jellyfish rather than higher-energy, more complex food webs (Greve & Parsons 1977, Purcell et al. 2007). This phenomenon may also have an effect on *P. quadrata* colonies, particularly in sea loch environments subject to nutrient inputs from agricultural runoff as well as aquacultural waste.

Any of these factors may play a role in *P. quadrata’s* appearance in Scottish waters. The summer timing of its abundance suggests that seasonally-governed environmental factors such as temperature, salinity and light may affect medusa production; however, since high-population density blooms of the medusae are infrequent, it can also be inferred that these conditions are not met constantly or even often. Therefore, none of the physical or biological factors listed above can yet be conclusively ruled out. Again, approaches for investigating the life cycle dynamics of *P. quadrata* are laid out in Part IX.

V. Forecasting a cnidarian bloom: recent investigations and projected strategies

Effective prediction of a harmful jellyfish bloom is an obvious objective for marine industry, but requires a far greater data bank than is currently available. Until the past decade, jellyfish have been of little economic interest, so there have been few historical surveys on which to base estimates of population flux. Several recent strategies for gathering data on gelatinous zooplankton occurrence in Scotland have been tested and implemented, with varying degrees of success. First, in a 2008 Crown Estate-funded survey of jellyfish impacts, aquaculture facility managers were provided with a standardized questionnaire regarding frequency, severity, and causative species via the Scottish Salmon Producers Organisation (SSPO). Of 257 potential respondents, only 9 responded (Nickell et al. 2010). One aquaculture syndicate in Shetland cited insurance concerns as a reason for withholding their data records. Obviously n = 9 is insufficient for meaningful data analysis, though it has been suggested that an increased timescale for completing the questionnaire might lead to an increased rate of response from other companies (Nickell et al. 2010). Remote sensing has also been investigated as a cnidarian research tool (Lynam et al. 2005, Nickell et al. 2010). Acoustic detection of jellyfish is possible for large medusae, though has not yet been put into broader-scale use as would be required for long-term population dynamic study, and is not yet applicable to smaller medusae such as *P. quadrata*. Aerial monitoring via spotter plane was trialled and found to be cost-prohibitive due to a combination of poor aerial visibility of problem species, and poor average weather conditions in the west of Scotland (Nickell et al., 2010). Remote sensing of blooms via satellite has been investigated but is restricted, again, by weather conditions and optical acuity (Nickell et al. 2010). Both aeroplane and satellite monitoring carry limitation when it comes to collecting data on *P. quadrata* appearance, for the basic reasons that the
medusae are small (under 15mm) and transparent (Russell 1953). Furthermore, bloom prediction by remote-sensing of jellyfish is effective only in detecting blooms that develop elsewhere and are carried by current flow; this might apply, for example, to the 2007 bloom of *Pelagia noctiluca* that moved in toward Northern Ireland and western Scotland, which could easily be sighted from the air before arriving inshore (Doyle et al. 2008). In the case of *P. quadrata*, both blooms discussed in the literature appear to have developed in the immediate vicinity of the salmon farms (Bruno and Ellis 1985, Seaton 1989, Ferguson et al. 2010); if this is accurate, aerial flyover or acoustic sampling data would be unable to provide any advance warning at all to afflicted farms.

Prediction of *Phialella quadrata* occurrence, therefore, might ideally be based on oceanographic and ecological factors leading to the mass release of medusae from the hydroid colony. This approach is contingent on gathering sufficient data to correlate bloom occurrence with environmental events. As very little data on *P. quadrata* is now publicly available, one approach to this might be combing through fish farm managers’ own logbooks to correlate dates of blooms with confirmed oceanographic and weather data. These correlations could be confirmed with an ongoing liaison with farms to collect data when and where new blooms occur. Early forays into this area have revealed that other species may co-occur during lower-level blooms, expanding the potential data available on medusa presence (Hope pers. obs. 2011). Additional information can come from life cycle data as discussed in Part III; any clues gleaned from historical *P. quadrata* appearances can be tested on laboratory cultures in order to evaluate their contributions to bloom production, as laid out in Part IX.

**VI. During a bloom: nematocysts, venom and infection**

As previously discussed, the 2008 *Phialella quadrata* bloom yielded information that fish were being affected in a two-part process: first, through envenoming by jellyfish nematocysts; and second, through infection by *T. maritimum*, transmitted from the jellyfish (Ferguson et al. 2010). These two separate means of pathology require separate consideration.

**A. Predatory ecology of the Cnidaria: the venom system**

All cnidarian classes at some point in the life cycle possess cnidocyte cells containing stinging organelles called cnidocysts. Cnidocysts can be divided into three groups: spirocysts, which are exclusive to the Anthozoa; ptychocysts, which are exclusive to the cerianthid anemones; and nematocysts, found across the phylum (Östman 2000). While spirocysts and ptychocysts are monotypic within their classification, nematocysts are diverse in type, placement on the body, structure, and predatory function (Hessinger & Lenhoff 1988, Östman 2000, Underwood and Seymour 2007). However, all are engaged in some form of delivery of venom and/or prey capture (Hessinger & Lenhoff 1988). Broadly speaking, nematocysts consist of a hollow capsule with a contained, inverted tubule which everts rapidly when stimulated by prey or self-defense to do so (Hessinger & Lenhoff 1988, Östman 2000). The predatory purpose of these tubules falls into two categories: those which entangle or stick to prey, and those which deliver venom (Hessinger & Lenhoff 1988). These were formerly termed astomocnidae and stomocnidae, in reference to a closed or open tubule tip that would allow for a flow of capsule contents (Weill 1934), but more
recent scanning electron microscopy images have found that nematocyst discharge often causes rupture of astomocnid tubules, and the terminology has been discarded (Östman 2000). However, for the purposes of discussing cnidarian predatory ecology, nematocysts can be viewed as penetrant or non-penetrant. Non-penetrants serve to tether or stick to microstructures on the outside of prey such as setae or exoskeletal plates, while penetrant nematocysts pierce prey integument either for venom delivery or for further tethering (Carrette et al. 2002, Hessinger & Lenhoff 1988).

The total complement of nematocysts (cnidome) and physical arrangement on the tentacles and bell varies taxonomically and ontogenetically, as does the toxin complement of contained venom (Carrette et al. 2002, Gravier-Bonnet 1987, Hessinger & Lenhoff 1988, Yanagihara et al. 2002, Underwood & Seymour 2007); therefore, differentiation between and within these types can help to discern the predatory habits, prey preferences, and ontogeny of the cnidarian in question (Carrette et al. 2002). In the context of Phialella quadrata ecology, a detailed knowledge of the hydroid and medusary cnidomes could contribute to estimates of prey capture capabilities and colonial growth potential, in turn aiding in predictions of bloom occurrence and magnitude. For example, if the P. quadrata hydroid cnidome is one best adapted to a specific type of zooplankton, it may follow that the local population of the same may correspond to medusa bloom magnitude in a given season.
Figure 2. Nematocyst organelles consist primarily of an enclosed capsule and a coiled, inverted tubule (a); appropriate stimuli will cause the tubule to evert rapidly (b), either stinging or entangling prey. (Nematocysts from *Carukia barnesi*, 1000x. Photograph J. Seymour 2002). Bands of nematocysts on *Chiropsella bronzei* (fm. *Chiropsalmus* sp.) tentacle shown at 100x (c) and 400x (d) show a diversity of types, including penetrant envenoming nematocysts and non-penetrant tethering and adhering nematocysts. (Tentacle photographs of *Chiropsella bronzei* by A. Kintner 2005). Nematocyst types may also vary according to ontogeny and placement on the body (e), with the proportion of nematocyst types on *Carukia barnesi* tentacles differing from those on the bell (Underwood and Seymour 2007) (Photograph by J. Seymour 2002.).

Meanwhile, there is virtually no information regarding the dynamics of *P. quadrata* venom and its contributions to fish kills during a bloom event. Ferguson et al. (2010) cite a variety of cnidarian venom mechanisms, but these run a very wide pharmacological and ecological gamut. Reported action of various hydrozoan and scyphozoan venoms has included neurotoxic pathways, type-specific muscle tissue signaling inhibition, enzymatic attack of epithelial tissues, various cytolytic pathways, and induction of pain-producing hormones such as histamines and serotonin (e.g. Tamkun & Hessinger 1981, Hessinger & Lenhoff 1988, Mustafa et al. 1995, Torres-Ramos & Aguilar 2003). Most of these studies show little species overlap of the molecular pathways leading to harmful outcomes, underlining the unique nature of each species’ venom regardless of taxonomic proximity. Further complications have been encountered as cnidarian venoms are notoriously difficult to study, contributing to an ongoing debate into preparation, preservation and purification of what can be considered genuine, ecologically relevant venom from the nematocysts (Bloom et al. 1998, Carrette & Seymour 2004, Kintner 2005). While a detailed investigation of venom mechanisms and valid extraction techniques is outside the
scope of this review, this list must demonstrate the futility of generalizing the actions of *P. quadrata* venom without at least a cursory investigation of its own particular actions.

Fortunately, the last decade has seen a number of studies which broaden the scope of cnidarian venoms investigation to include ecological rather than only pharmacological contexts, with many toxin pathways being linked to prey preference and niche (Carrette et al. 2002, Torres-Ramos & Aguilar 2003, Kintner et al. 2005). Many examples of neurotoxicity, for example, have been shown to operate most efficiently in crustacean models and can be reproduced only poorly in vertebrate models (Torres-Ramos & Aguilar 2003), demonstrating prey-specific targeting of venom compounds. Cofamiliars with widely different prey targets may exhibit a number of different venom toxin assemblages according to prey habit (Carrette et al. 2002, Kintner et al. 2005). It is possible that venom toxin complement may, like the cnidome, vary ontogenetically as prey preference and environmental conditions change.

Consideration of these dynamics may assist in developing mitigation strategies when *P. quadrata* blooms occur. First, as with the cnidome, an understanding of venom ontogeny can help predict prey ecology and resource availability for both pelagic medusae and benthic colonies. Second, a basic understanding of toxicity may help in risk assessment of bloom magnitude—e.g., small or dispersed blooms may be unable to deliver critical damage to fish, and can be calculated as such to avoid unnecessary disruption of normal farm operation. Third, knowing the proportionate damage of venom vs. bacterial infection would help to devise treatments for affected fish pens that minimize side effects and environmental impact of chemical treatments. A procedure for investigating basic characteristics of *P. quadrata* venom using cultured gill cells is given in Part IX.

**B. Bacterial infection and symbiosis**

The final aspect of *P. quadrata* bloom biology to consider is the role of *Tenacibaculum maritimum* infection. A major component of fish morbidity is attributable to *T. maritimum* action in the gill tissues (Ferguson et al. 2010). *T. maritimum* is a relatively well-known fish pathogen and is most often linked to skin conditions, particularly in farmed fish, though tenacibaculosis of the gills is by no means rare (Avendaño-Herrera et al. 2006). Occasionally the bacilli have been found living in fish dermal mucus, suggesting the possibility of their being endemic there (Avendano-Herrera et al. 2005). Given this situation, it has been suggested that the jellyfish may be picking up the bacteria from fish skins and merely transferring them to gill tissue as the medusae are sucked in through the mouths of salmon; however, tenacibaculosis in any form is virtually unheard of in Shetland salmon, including as a dermal pathology (Sutherland pers. obs. 2012). This does not rule out the fish themselves as the origin of bacteria, but certainly argues against the idea. Moreover, no other natural, non-pathogenic reservoirs of *T. maritimum* are yet known (Avendaño-Herrera et al. 2006). Scanning electron microscopy of *P. quadrata* medusae showed a heavy colonization of the bacteria on the mouth parts (Ferguson et al., 2010). Therefore, the question of a long-term symbiosis between the two species should be considered.

Close microbial symbiosis in cnidarians is well known, particularly in the cases of shallow water hard corals and various scyphozoans such as the *Cassiopeia* and *Mastigias* genera, all of which harbour photosynthetic zooxanthellae. However, the *P. quadrata* bloom in Shetland marked the first recorded
instance of a medusa symbiosis with a prokaryotic organism (Ferguson et al., 2010). A similar symbiosis was found in the past year, with *T. maritimum* living on the mouth parts of *Pelagia noctiluca* medusae which had had no contact with farmed fish (Ferguson et al., 2010). This lends credence to the possibility that the relationship between medusa and bacteria is long term and not merely a case of pathogen transference from skin to gills.

![Figure 3](image)

**Figure 3.** Microbial symbiosis is well-known in the cnidarian phylum, but until recently, only eukaryotic algal symbionts have been found. These include the photosynthesizing zooxanthellae of two scyphozoan genera: the “upside-down” jellyfish of the *Cassiopeia* genus (a) (Photograph T. Laman), the *Mastigias* sp. medusae best known from enclosed lakes in Palau (b) (photograph T. Laman) and the perhaps best-known photosynthetic zooxanthellae of anthozoan hard corals (c) (photograph B. Skerry). In the scyphozoans, nematocyst complement may be reduced or altered in the presence of the zooxanthellae, with the jellyfish dependent on photosynthetically-derived energy.

If medusae are living symbiotically with marine bacteria, this raises the question of the nature of the symbiosis: parasitic, wherein the bacteria are pathogens of the medusae themselves; commensal, wherein the bacteria are incidental to the survival and success of the medusae but benefit from a free ride to whatever organisms the jellyfish may be preying on; or mutualistic, wherein the bacteria provide some kind of selective advantage to the jellyfish and vice versa. This latter possibility might recall the gut flora mutualism in higher animals, wherein endosymbiotic bacteria would provide digestive and enhanced nutritional benefit to the medusae. Furthermore, if any form of symbiosis is long-term, it is not known at what point in the hydroid life cycle the bacteria become involved, whether at the colonial hydroid stage or exclusively during the pelagic medusa stage. Bacterial contributions (or detriments) to *P. quadrata* reproductive fitness and survival and role in the various life stages should be pertinent lines of inquiry into further study of bloom ecology. These investigations should dovetail with other hydroid culture experiments already being undertaken, as described in Part IX.

**VII. Conclusion and proposed investigative avenues**

The present state of knowledge regarding *Phialella quadrata*’s involvement with the Scottish aquaculture industry is inadequate to begin developing mitigation strategies. The primary goal of my studies for the degree of Doctor of Philosophy in Marine Biology will be to provide information towards these strategies and toward new questions in ecology raised by *P. quadrata* bloom events. First, *P. quadrata* hydroid colony ecology will be examined, including the growth and polyp specialization determinants. Environmental stimuli leading to the generation and release of medusae, and their maturation and lifespan in the plankton will also be investigated. These lines of inquiry can be followed
by a simultaneous approach with experimental laboratory culture of the hydroids, plus a collation of historical data on the appearance and blooms of *P. quadrata* medusae. Second, the role of the venoms system, including the cnidome and basic information on the venom itself, will be explored in order to better inform the predator-prey relationship and predatory strategy. Finally, a characterization of the relationship between *P. quadrata* and *Tenacibaculum maritimum* will be undertaken. These latter goals can be accomplished in concert with hydroid colonial rearing, plus sufficient access to wild *P. quadrata* medusae. These investigations will hopefully lead to an informed and effective approach in predicting and treating future blooms of *P. quadrata*, and add to the body of knowledge of gelatinous zooplankton ecology.

**VIII. References**


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Laman, T. “Crab carrying jellyfish.” Copyright: T. Laman/National Geographic.
Laman, T. "Mastigias sp." Copyright: T. Laman/National Geographic.

Link, Otto. *Phialella quadrata* medusa. Photograph courtesy of Scottish Association for Marine Sciences, Oban, Scotland.


Seymour, J. Unfired and fired nematocysts of *Carukia barnesi* at 400x magnification. James Cook University of North Queensland, Australia. Reprinted with permission.


Skerry, B. "National Geographic: Hard coral carpets a shallow seafloor on Kingman Reef." Copyright: National Geographic/Brian J. Skerry.


IX. Appendix: Intended procedures for data collection and analysis

This project will consider several aspects of *Phialella quadrata* biology, toward the stated goals of prediction and mitigation of harmful blooms. Research objectives include:

1) Identifying environmental factors governing the presence of *P. quadrata* medusae, with a view to using these to develop predictive protocols;
2) Examining *P. quadrata* venoms ecology and aetiology of gill injury at salmon farms, through examination of the cnidome, prey analysis, and basic venom characterization;
3) Defining the nature of *P. quadrata* symbiosis with the bacterium *Tenacibaculum maritimum*.

Procedural plans for Objective 1: “Identification of environmental factors governing the presence of *P. quadrata* medusae.” This will be accomplished jointly through (a) collection of historical data and (b) laboratory culture of *P. quadrata*.

(a) Historical data will come from logged zooplankton appearances in aquaculture industry records. To this end, I am coordinating with Rachel Hope of Hjaltland Sea Farms in Lerwick, Shetland, and Christopher Wallace of Marine Harvest Scotland, to contact and gather information from these sources. Records of *P. quadrata* presence will be collated according to site, season, population density (e.g. whether the animals are swarming in high numbers or are a low-level presence in a zooplankton community), and oceanographic/meteorological data for a reasonable preceding timeframe. This will result in a database of *P. quadrata* occurrence, hopefully dating from at least the late 1990s and giving weekly or biweekly notes of the zooplankton community, though this will undoubtedly vary from source to source. If sufficient data is available, retrospective factorial analysis will be used to identify conditions contributing to swarm appearance. I will aim to begin this aspect of research over the 2012 winter.

(b) Laboratory culture of *P. quadrata* will commence this summer. As many medusae as possible will be collected, from beach sites near the Scottish Association for Marine Sciences in Dunstaffnage, from pier sites in Vidlin Voe in Shetland, and (should a major bloom event occur) from any affected aquaculture sites. The latter factor will depend on participation of the salmon farming industry; as mentioned in part (a), both Hjaltland Sea Farms and Marine Harvest have expressed interest in the project, and are helping to facilitate on-site contacts who will contact me in the event of an obvious bloom and/or a fish kill with similar signs as occurred during the 2008 *P. quadrata* bloom in Shetland. Some captured medusae will be brought to research facilities at SAMS and induced to spawn; others will be kept for use in Objective 2 (see below). Planula larvae will be placed in a culture dish and allowed to settle and form a stable attachment to substrate for several days before regular feeding with *Artemia* nauplii commences. Established colonies will be divided into separate culture plates for experimental investigation.

A pilot study examining effects of temperature and salinity using a two-way factorial ANOVA design will be undertaken first in order to establish a baseline for medusa production. Results from this study, plus information gleaned from the historical data culled above, will contribute to design of a more comprehensive set of experimental comparisons using more
variables and examining both medusa production and colonial expansion (as measured by wet biomass), using MANCOVA to analyse whether chosen variables are having a significant effect, and whether colonies are allocating resources toward expansion, medusa production, or a combination of the two. Variables to be tested will be selected based on Part (a), but may include temperature, salinity, and food availability. Following this, the role of hormesis will be investigated using new hydroid colonies held at in steady-state controlled conditions, followed by a spike in a single factor (e.g. a rapid rise or fall in temperature). These will be analyzed using single-factor ANOVA comparison to a negative control colony.

I will aim to complete the pilot study aspect of research, plus develop sufficient stock cultures of *P. quadrata* hydroids for the second phase of experiments, over the summer of 2012 at SAMS. Follow-up experiments of more detailed environmental conditions will be conducted at SOI using equipment already in use for similar procedures.

(c) Field surveys will be conducted in order to identify natural and manmade substrates hosting *P. quadrata* colonies. This will be accomplished through collecting specimens from fouling communities on aquaculture infrastructure. Hydroids obtained will be reared and identified; if *P. quadrata* or its congeners are present, a field-settlement study examining preferred substrate, preferred depth, and natural vs. artificial surfaces will be undertaken. Sampling may commence in early spring of 2012 in Vidlin Voe, Shetland. Depending on outcomes, experimental field settlement studies will likely take place in summer of 2013.

**Procedural plans for Objective 2:** “Venom ecology of *P. quadrata*: cnidome, prey analysis, and basic venom characterization.”

*P. quadrata* venom will be collected from live medusae according to methods set out by Bloom et al. (1998) and Carrette & Seymour (2002), involving tentacle decay and nematocyst lyophilisation for storage, then extraction from rehydrated nematocysts when required. SDS-PAGE gel electrophoresis will be used to obtain a profile of the number and molecular weights of the proteins involved. Toxicity will be assessed using cultures of the established gill cell line of RTgill-W1 rainbow trout lamellar tissue after Helmholtz et al. (2010), using Sytox Green immunofluorescence reagent to indicate cell death after treatment with venom doses. Results will be analysed using linear regression as per Kintner et al. (2005). These studies will be conducted during the winter of 2013, after a sufficient source of venom have been collected and stored from source medusae. Facilities and supervision for these procedures have been made available by Dr Val Smith at SOI.

**Procedural plans for Objective 3:** “Defining the nature of *P. quadrata* symbiosis with the bacterium *Tenacibaculum maritimum*.”

The relationship between *P. quadrata* and *T. maritimum* will be investigated through several avenues of inquiry. First, *P. quadrata* medusae taken from non-salmon farm sites, e.g. those not involved in harmful blooms, will be sampled for the presence of *T. maritimum* in order to assess
the incidence of symbiosis outwith interaction with caged salmon. This will take place simultaneously with medusa collection for culture (e.g. summers of 2012 and 2013). Cultured and wild *P. quadrata* colonies, as well as medusae released during experiments, will also be sampled. Any strains of *T. maritimum* found will be compared to strains found on fish skins during medusa bloom events. These studies will be carried out at the Moredun Research Institute in Edinburgh, in conjunction with Dr Christian Delannoy. Should *T. maritimum* be found in conjunction with *P. quadrata* colonies, an attempt will be made to raise a sterile, asymbiotic hydroid colony, in order to compare relative success of colonial growth and reproductive success in the presence and absence of bacteria. Analysis of outcomes will be carried out using one-way ANOVA. Should *P. quadrata* be found to acquire *T. maritimum* only as hydromedusae, medusa success in the presence and absence of bacteria will be compared by assessing time to gamete release and overall lifespan between *T. maritimum*-positive and *T. maritimum*-negative medusae (to be compared using $\chi^2$ analysis). This approach will establish whether *T. maritimum* inoculation is helpful, harmful, or neutral to *P. quadrata* in its various life phases.