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Review Article

# Rickettsial and Chlamydial Infections of Freshwater and Marine Fishes, Bivalves, and Crustaceans

John L. Fryer[1] and Catharine N. Lannan[2]

[1]*Department of Microbiology, Oregon State University, Nash Hall 220, Corvallis, Oregon 97331-3804, USA*
[2]*Laboratory for Fish Disease Research, Department of Microbiology, Oregon State University, Mark O. Hatfield Marine Science Center, Newport, Oregon 97365-5296, USA*

## CONTENTS



## ABSTRACT

**John L. Fryer and Catharine N. Lannan (1994)** Rickettsial and chlamydial infections of freshwater and marine fishes, bivalves, and crustaceans. *Zoological Studies* **33**(2): 95-107. Intracellular, gram-negative, procaryotic organisms infect a wide range of aquatic poikilotherms. Both pathogenic and benign infections are observed, and occasionally, these intracellular bacteria are highly virulent. Rickettsial and chlamydial taxonomy has not been clearly established, but the agents infecting aquatic poikilotherms are commonly described as rickettsia-like or chlamydia-like, based on morphology and the presence or absence of pleomorphic development stages as observed by light and electron microscopy. Only two have been assigned at the species level, and only one has been isolated and characterized in vitro. Isolation and culture of these intracellular pathogens is difficult, due both to a lack of appropriate culture systems and the presence of contaminating organisms in or on host tissues used for inoculation. A complete understanding of the intracellular bacteria infecting aquatic poikilotherms and determination of their relationships to each other and to described rickettsial and chlamydial species is dependent on the development of techniques for laboratory maintenance and culture, not only of these rickettsia-like and chlamydia-like organisms, but also, in some cases, of their aquatic hosts.

**Key words:** Rickettsia, Chlamydia, Fish, Bivalves, Crustaceans.

## INTRODUCTION

Current literature reflects a large and diverse group of gram-negative, intracellular procaryotic organisms infecting aquatic poikilotherms. The majority of these infections are in marine or anadromous hosts, but they also occur in animals who spend their entire life cycle in the freshwater environment (Federici et al. 1974, Molnar and Boros 1981, Paperna et al. 1981, Larsson 1982, Zimmer et al. 1984, Bradley et al. 1988, Desser et al. 1988). Only one of these procaryotic organisms has been isolated and characterized in vitro, most have been described only by light and electron microscopy. Although they are commonly termed rickettsia-like or chlamydia-like, their precise taxonomic placement has not been determined.

The taxonomy of the gram-negative, intracellular bacteria is unclear, although the monogeneric order, Chlamydiales, is closely defined (Moulder 1984). The described chlamydial species are phenotypically similar. All are coccoid; they replicate within cytoplasmic vacuoles in host cells in a characteristic pleomorphic development cycle that alternates between a rigid-walled infectious form and a flexible-walled replicative form; and they share a genus-specific, lipopolysaccharide antigen.

The remaining intracellular bacteria have been placed in the order, *Rickettsiales* (Weiss and Moulder 1984), making the rickettsiae a highly diverse group. Binary fission is common to all, but exceptions exist to each of the other characteristics attributed to them (Weiss and Moulder 1984). They may replicate within vacuoles or free in the nucleus or cytoplasm of host cells. Most are rod-shaped, but many are coccoid or pleomorphic. Most are non-motile, but flagellated forms are included. Most do not exhibit a pleomorphic life cycle, but this type of development is found in the genus *Rickettsiella*. Most are transmitted via an alternate host or vector, but *Coxiella burnetii* can be transmitted directly. Most are obligate intracellular parasites, but species of the genus *Rochalimaea* can be cultured on host cell-free media.

When more information is available on the taxonomic relationships of the intracellular bacteria, they will undoubtedly be further divided. In the interim, those infecting aquatic poikilotherms will continue to be considered rickettsia-like or chlamydia-like based on morphology and the observed presence or absence of a pleomorphic development cycle.

## FISHES

Chlamydia-like organisms have been observed in at least thirty-five fish species from both the freshwater and the marine environment (Table 1). None of these chlamydia-like organisms has been isolated, and characterization of them has been limited to description of their cellular morphology and study of the pathology they produce in infected hosts.

All of these chlamydia-like organisms of fishes are associated with epitheliocystis disease, a widely reported infection that occurs in either proliferative or benign form and is characterized by cysts in the branchial epithelium of the host fish (Fig. 1). The cysts are greatly hypertrophied host cells filled with the procaryotic agents. In many cases, the infection elicits little host response, but at times and under circumstances not clearly understood, extensive hyperplasia of the gill epithelium is induced. When this occurs, the resulting lamellar fusion and clubbing seriously impair oxygen uptake and osmoregulatory processes in the host and frequently lead to death.

Epitheliocystis was first described in the bluegill (*Lepomis macrochirus*), by Hoffman et al. (1969), who suggested a bedsonia-like (chlamydia-like) etiology. Earlier, a similar disease of common carp (*Cyprinus carpio*) was described by Plehn (1920), who termed the disease, mucophilosis, and attributed it to an algal cause. Molnar and Boros (1981) determined that epitheliocystis and mucophilosis were indistinguishable and verified the chlamydia-like nature of the etiologic agent. Subsequently, epitheliocystis has been observed throughout the temperate zone in freshwater, anadromous, and marine fish species and in both warmwater and coldwater environments (Table 1).

Although the precise taxonomic placement of the morphologically diverse, intracellular, procaryotic epitheliocystis organisms has not been determined, they usually are considered to fit best in the order *Chlamydiales* (Moulder 1984). Most range in diameter from ca. 0.2 to 1.2 $\mu$m, have a typical gram-negative cell wall, and exhibit the pleomorphic development cycle that is characteristic of the chlamydiae. A coccoid to ovate form is common to all, but chains (Desser et al. 1988) and branched (Paperna et al. 1978), elongated (Paperna et al. 1981), or tailed forms (Rourke et al. 1984, Bradley et al. 1988) have been observed, and a high degree of host specificity exists (Paperna 1977).

Unlike the widespread chlamydia-like infections of fish, rickettsia-like organisms in fish have been less frequently reported (Table 2). In 1939, Mohamed noted a coccoid rickettsia-like agent within monocytes and in plasma of one dead tetrodontid fish in Egypt (Wolf 1981). No further reports of this organism or of other fish rickettsiae occurred until Ozel and Schwanz-Pfitzner (1975) detected a rod-shaped rickettsia-like agent in tissues of rainbow trout (*Oncorhynchus mykiss*) collected from a freshwater source in Europe. The agent was isolated and passed in fish cell cultures, but it was not characterized beyond morphological description, nor was it maintained for further study. Viral Hemorrhagic Septicemia Virus was also isolated from the affected trout, so although mortality was observed in the trout population, the role of the rickettsia-like agent in the observed mortality is undetermined. In 1986, Davies (1986) reported a "coccoid or elongate" rickettsia-like organism, observed by transmission electron microscopy, in tissues of the dragonet (*Callionymus lyra*), a marine fish collected in Cardigan Bay, Wales.

**Table 1.** Epitheliocystis (a Chlamydia-like Infection) in Fishes

| Host Species[1] | Geographic Region | Reference |
|---|---|---|
| ACIPENSERIDAE | | |
| *Acipenser transmontanus* (White sturgeon) | California USA | unpublished |
| CARANGIDAE | | |
| *Seriola dumerili* (Amberjack) | Western Mediterranean Sea | Crespo et al. (1990)<br>Grau & Crespo (1991) |
| CENTRARCHIDAE | | |
| *Lepomis macrochirus* (Bluegill) | West Virginia USA | Hoffman et al. (1969) |
| CICHLIDAE | | |
| *Tilapia aurea x nilotica* (Blue/Nile tilapia hybrid) | Israel | Paperna et al. (1981) |
| *Tilapia mossambica* (Mozambique tilapia) | South Africa, Israel<br>South Africa | Paperna & Sabnai (1980)<br>Paperna et al. (1981) |
| *Tilapia nilotica* (Nile tilapia) | Israel | Paperna & Sabnai (1980) |
| CYPRINIDAE | | |
| *Cyprinus carpio* (Common carp) | Europe<br><br>Israel, Portugal<br>Mie Prefecture Japan | Plehn (1920)<br>Molnar & Boros (1981)<br>Paperna & Alves de Matos (1984)<br>Miyazaki et al. (1986) |
| GADIDAE | | |
| *Gadus morhua* (Atlantic cod) | Northwest Atlantic Ocean | Lewis et al. (1992) |
| ICTALURIDAE | | |
| *Ictalurus nebulosus* (Brown bullhead) | Lake Ontario Canada | Desser et al. (1988) |
| *Ictalurus punctatus* (Channel catfish) | Oklahoma USA | Zimmer et al. (1984) |
| MORONIDAE | | |
| *Morone americanus* (White perch) | Connecticut USA | Wolke et al. (1970) |
| *Morone saxatalis* (Striped bass) | Connecticut, USA<br>Chesapeake Bay USA | Wolke et al. (1970)<br>Paperna & Zwerner (1976) |
| *Dicentrarchus labrax* (Sea bass) | Mediterranean Coast, France<br>Mediterranean and Atlantic Coast, France | Paperna & Baudin Laurencin (1979)<br>Paperna et al. (1981) |
| MUGILIDAE | | |
| *Liza aurata* | Eastern Mediterranean & Red Sea<br>Israel | Paperna & Sabnai (1980)<br>Paperna et al. (1981) |
| *Liza ramada* | Eastern Mediterranean & Red Sea<br>Israel | Paperna (1977)<br>Paperna et al. (1978)<br>Paperna et al. (1981) |
| *Liza subviridis* | Eastern Mediterranean & Red Sea | Paperna & Sabnai (1980) |
| *Mugil cephalus* (Striped mullet) | Eastern Mediterranean & Red Sea<br>Israel | Paperna & Sabnai (1980)<br>Paperna et al. (1981) |
| MULLIDAE | | |
| *Upeneus mollucensis* (Goatfish) | Eastern Mediterranean Sea | Paperna & Sabnai (1980) |

**Table 1.** (cont.)

| Host Species[1] | Geographic Region | Reference |
|---|---|---|
| OPLEGNATHIDAE | | |
| *Oplegnathus punctatus* (Rock sea bream) | Japan | Egusa et al. (1987) |
| PARALICHTHYIDAE | | |
| *Paralichthys dentatus* (Summer flounder) | Northwest Atlantic Ocean | Lewis et al. (1992) |
| *Paralichthys oblongus* (Fourspot flounder) | Northwest Atlantic Ocean | Lewis et al. (1992) |
| PHYCIDAE | | |
| *Urophycis regia* (Spotted hake) | Northwest Atlantic Ocean | Lewis et al. (1992)<br>Zachary & Paperna (1977) |
| PLEURONECTIDAE | | |
| *Hippoglossoides platessoides* (American plaice) | Northwest Atlantic Ocean | Morrison & Shum (1983a) |
| *Pleuronectes americanus* (Winter flounder) | Northwest Atlantic Ocean | Lewis et al. (1992) |
| *Pleuronectes ferrugineus* (Yellowtail flounder) | Northwest Atlantic Ocean | Lewis et al. (1992) |
| SALMONIDAE | | |
| *Oncorhynchus mykiss* (Steelhead trout) | Idaho USA | Rourke et al. (1984) |
| *Salvelinus namaycush* (Lake trout) | Great Lakes USA | Bradley et al. (1988) |
| SCIAENIDAE | | |
| *Cynoscion regalis* (Weakfish) | Northwest Atlantic Ocean | Lewis et al. (1992) |
| SCOPHTHALMIDAE | | |
| *Scophthalmus aquosus* (Windowpane) | Northwest Atlantic Ocean | Lewis et al. (1992) |
| SPARIDAE | | |
| *Acanthopagrus schlegeli* (Black sea bream) | Japan | Egusa (1987) |
| *Pagrus major* (Red sea bream) | Hong Kong/Japan | Miyazaki et al. (1986) |
| *Sparus aurata* (Gilthead sea bream) | Eastern Mediterranean & Red Sea<br>Israel<br>Mediterranean Coast France | Paperna (1977)<br>Paperna et al. (1978)<br>Paperna & Baudin Laurencin (1979) |
| STROMATEIDAE | | |
| *Peprilus triacanthus* (Butterfish) | Northwest Atlantic Ocean | Lewis et al. (1992) |
| SYGNATHIDAE | | |
| *Phycodurus eques* (Leafy sea dragon) | Australia | Langdon et al. (1991) |
| TETRAODONTIDAE | | |
| *Takifugu rubripres* (Tiger puffer) | Kagoshima Prefecture Japan | Miyazaki et al. (1986) |
| ZOARCIDAE | | |
| *Macrozoarces americanus* (Ocean pout) | Northwest Atlantic Ocean | Lewis et al. (1992) |

[1]classification as Eschmeyer (1990).

Fig. 1. Epitheliocystis cyst in the gill of a juvenile white sturgeon (*Acipenser transmontanus*). Hematoxylin and eosin stain. Bar = 100 $\mu$m.

No rickettsia-like organism from fish was characterized sufficiently for precise taxonomic placement until Fryer and coworkers (1990) isolated an agent of this type from diseased coho salmon (*Oncorhynchus kisutch*) collected in Chile from a seawater netpen where an epizootic was in progress. Through in vitro characterization and 16S rRNA analysis, they established its placement in a new genus and species (*Piscirickettsia salmonis* gen. nov., sp. nov., Type strain LF-89) within the order Rickettsiales and the family Rickettsiaceae (Fryer et al. 1992). The isolated agent is pleomorphic but predominantly coccoid, ca. 0.5-1.5 $\mu$m in diameter, and it replicates within membrane-bound cytoplasmic vacuoles in fish tissues and in certain fish cell lines (Fig. 2). In vitro replication of *P.*

**Table 2.** Rickettsial and Rickettsia-like Infections of Fishes

| Host Species[1] | Geographic Region | Reference |
|---|---|---|
| CALLIONYMIDAE | | |
| *Callionymus lyra* (Dragonet) | Cardigan Bay Wales | Davies (1986) |
| SALMONIDAE | | |
| *Oncorhynchus kisutch* (Coho salmon) | Southern Coast Chile | Fryer et al. (1990, 1992)<br>Branson & Diaz-Munoz (1991)<br>Cvitanich et al. (1991)<br>Garcés et al. (1991)<br>Lannan et al. (1991)<br>Fryer & Lannan (1992) |
| *Oncorhynchus mykiss* (Rainbow trout) | Germany<br>Southern Coast Chile | Ozel & Schwanz-Pfitzner (1975)<br>Cvitanich et al. (1991)<br>Garcés et al. (1991)<br>Fryer et al. (1992) |
| *Oncorhynchus tshawytscha* (Chinook salmon) | Southern Coast Chile | Cvitanich et al. (1991)<br>Garcés et al. (1991)<br>Fryer et al. (1992) |
| | Pacific Coast Canada | Evelyn (1992) |
| *Oncorhynchus gorbuscha* (Pink salmon) | Pacific Coast Canada | Evelyn (1992) |
| *Salmo salar* (Atlantic salmon) | Southern Coast Chile | Cvitanich et al. (1991)<br>Garcés et al. (1991)<br>Fryer et al. (1992) |
| | Pacific Coast Canada | Evelyn (1992) |
| TETRAODONTIDAE | | |
| Unknown | Egypt | Mohamed (1939) |

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| PARALICHTHYIDAE | | |
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| *Paralichthys oblongus* (Fourspot flounder) | Northwest Atlantic Ocean | Lewis et al. (1992) |
| PHYCIDAE | | |
| *Urophycis regia* (Spotted hake) | Northwest Atlantic Ocean | Lewis et al. (1992)<br>Zachary & Paperna (1977) |
| PLEURONECTIDAE | | |
| *Hippoglossoides platessoides* (American plaice) | Northwest Atlantic Ocean | Morrison & Shum (1983a) |
| *Pleuronectes americanus* (Winter flounder) | Northwest Atlantic Ocean | Lewis et al. (1992) |
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| SALMONIDAE | | |
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| SCIAENIDAE | | |
| *Cynoscion regalis* (Weakfish) | Northwest Atlantic Ocean | Lewis et al. (1992) |
| SCOPHTHALMIDAE | | |
| *Scophthalmus aquosus* (Windowpane) | Northwest Atlantic Ocean | Lewis et al. (1992) |
| SPARIDAE | | |
| *Acanthopagrus schlegeli* (Black sea bream) | Japan | Egusa (1987) |
| *Pagrus major* (Red sea bream) | Hong Kong/Japan | Miyazaki et al. (1986) |
| *Sparus aurata* (Gilthead sea bream) | Eastern Mediterranean & Red Sea<br>Israel<br>Mediterranean Coast France | Paperna (1977)<br>Paperna et al. (1978)<br>Paperna & Baudin Laurencin (1979) |
| STROMATEIDAE | | |
| *Peprilus triacanthus* (Butterfish) | Northwest Atlantic Ocean | Lewis et al. (1992) |
| SYGNATHIDAE | | |
| *Phycodurus eques* (Leafy sea dragon) | Australia | Langdon et al. (1991) |
| TETRAODONTIDAE | | |
| *Takifugu rubripres* (Tiger puffer) | Kagoshima Prefecture Japan | Miyazaki et al. (1986) |
| ZOARCIDAE | | |
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**Fig. 1.** Epitheliocystis cyst in the gill of a juvenile white sturgeon (*Acipenser transmontanus*). Hematoxylin and eosin stain. Bar = 100 μm.

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| SALMONIDAE | | |
| *Oncorhynchus kisutch* (Coho salmon) | Southern Coast Chile | Fryer et al. (1990, 1992)<br>Branson & Diaz-Munoz (1991)<br>Cvitanich et al. (1991)<br>Garcés et al. (1991)<br>Lannan et al. (1991)<br>Fryer & Lannan (1992) |
| *Oncorhynchus mykiss* (Rainbow trout) | Germany | Ozel & Schwanz-Pfitzner (1975) |
| | Southern Coast Chile | Cvitanich et al. (1991)<br>Garcés et al. (1991)<br>Fryer et al. (1992) |
| *Oncorhynchus tshawytscha* (Chinook salmon) | Southern Coast Chile | Cvitanich et al. (1991)<br>Garcés et al. (1991)<br>Fryer et al. (1992) |
| | Pacific Coast Canada | Evelyn (1992) |
| *Oncorhynchus gorbuscha* (Pink salmon) | Pacific Coast Canada | Evelyn (1992) |
| *Salmo salar* (Atlantic salmon) | Southern Coast Chile | Cvitanich et al. (1991)<br>Garcés et al. (1991)<br>Fryer et al. (1992) |
| | Pacific Coast Canada | Evelyn (1992) |
| TETRAODONTIDAE | | |
| Unknown | Egypt | Mohamed (1939) |

[1]classification as Eschmeyer (1990).

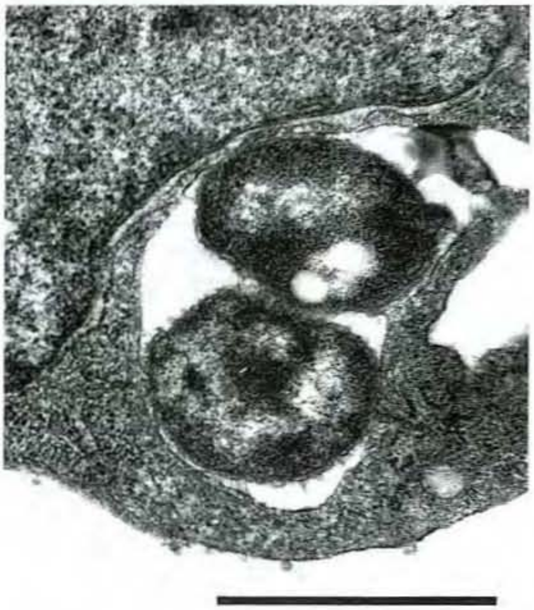

Fig. 2. Transmission electron micrograph of *Piscirickettsia salmonis* within a cytoplasmic vacuole in a cultured CHSE-214 cell. Bar = 1 μm.

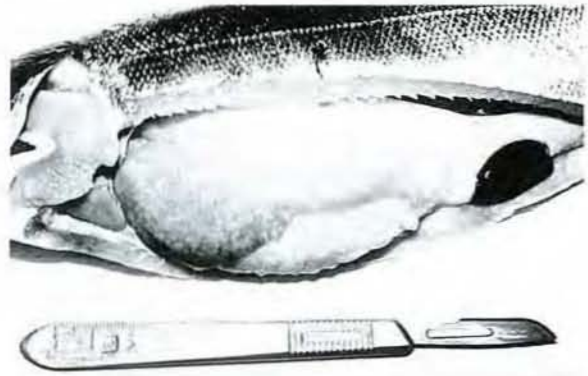

Fig. 3. Gross pathology associated with piscirickettsiosis in coho salmon (*Oncorhynchus kisutch*). The kidney is swollen, the spleen enlarged, and the gill is pale in color. Note the mottled liver which is characteristic of the chronic form of this disease.

*salmonis* is optimal between 15 and 18°C. It is sensitive in vitro to a wide range of antibiotics, but not to penicillin. Garcés et al. (1991) demonstrated the pathogenicity and virulence of the type strain for both coho and Atlantic salmon (*Salmo salar*).

Since *P. salmonis* was characterized, several morphologically similar agents have been detected in salmonid fish at widely-spaced locations around the world. One of these organisms was identified in 1991, in diseased Atlantic salmon collected from a seawater farm in British Columbia, Canada (Evelyn 1992). A similar, but unidentified, organism reportedly induced mortality as early as 1970 in pink salmon (*Oncorhynchus gorbuscha*) held in seawater tanks in the region (Evelyn 1992). The agent observed in Atlantic salmon shares one or more common antigen(s) with *P. salmonis*, but it is apparently less virulent than the type strain, LF-89. Another rickettsial organism was detected in Norway in histological sections of liver from Atlantic salmon experiencing a disease termed necrotizing hepatitis (T. Hastein, National Veterinary Institute, Oslo, personal communication 1992). An organism of this type was also observed in electron micrographs of tissues of Atlantic salmon reared in Ireland (H. Rodger, University of Stirling, Scotland, personal communication 1992). All of these rickettsia-like organisms produce pathology similar to that associated with piscirickettsiosis in Chilean salmonids (Fig. 3), but their precise relationships to *P. salmonis* and to each other have not been determined.

## MOLLUSCS

Intracellular procaryotic organisms have been observed in at least 25 species of marine bivalves (Table 3). These infections are frequently benign in adult animals, although the stress resulting from a heavy infectious load likely has a deleterious effect on the overall health of the host. Extensive mortality is occasionally noted concurrent with the infections in marine bivalves, but often environmental stress, e.g. winter low temperatures (Gulka et al. 1983, Le Gall et al. 1991), may be a contributing factor. In addition, the developmental stage of the host may also be an important element affecting the outcome of the infection. Leibovitz (1989) reported a chlamydia-like agent that was highly virulent for larval and postmetamorphic stages of the bay scallop (*Argopecten irradiens*) but was avirulent for adult animals.

The digestive gland and the gills of marine bivalves are the organs most frequently affected. Granular, basophilic, intracytoplasmic inclusions or plaques are characteristically found in the infected tissues (Fig. 4). The plaques are frequently described as Fuelgen-positive, and usually no apparent host response is elicited.

A diverse group of microorganisms is respon-

**Table 3.** Rickettsia-like and Chlamydia-like Infections of Marine Bivalves

| Host Species | Geographic Region | R/C[1] | Reference |
|---|---|---|---|
| *Argopecten irradiens* (Bay scallop) | Prince Edward Island Canada | C | Morrison & Shum (1982) |
| | | R | Morrison & Shum (1983b) |
| | NE Atlantic Coast USA | R | Leibovitz et al. (1984) |
| | | C | Leibovitz (1989) |
| *Chlamys varia* (scallop) | — | R | LeGall et al. (1991b) |
| *Chlamys opercularis* (scallop) | — | R | LeGall et al. (1991b) |
| *Cerastoderma edule* | Brittany France | R or C | Auffret & Poder (1987) |
| *Crassostrea angulata* (Portuguese oyster) | — | R or C | Comps & Deltreil (1979) |
| *Crassostrea gigas* (Pacific oyster) | France | R | Comps et al. (1977a,b) |
| | Alaska, USA | R | Meyers et al. (1990) |
| | Atlantic Coast Spain | R | Azevedo & Villalba (1991) |
| *Crassostrea virginica* (Eastern oyster) | New York, USA | R | Meyers (1981) |
| | Gulf Coast, USA | R | Couch (1985) |
| *Donax trunculus* (Wedge shell) | Mediterranean Coast France | R | Comps (1985b) |
| *Mercenaria mercenaria* (Northern quahog) | Chesapeake Bay USA | C | Otto et al. (1977) |
| | | C | Harshbarger et al. (1977) |
| | | C | Page & Cutlip (1982) |
| | New York, USA | C | Meyers (1979) |
| | | R & C | Meyers (1981) |
| | Delaware, USA | R | Fries & Grant (1991, 1992) |
| *Mya arenaria* (Softshell clam) | Chesapeake Bay USA | R | Harshbarger et al. (1977) |
| | | R | Otto et al. (1977) |
| | Delaware, USA | R | Fries et al. (1991) |
| *Mytilus californianis* (Californis mussel) | Pacific Coast USA | R or C | Yevich & Barszcz (1983) |
| *Mytilus edulis* (Blue mussel) | Rhode Island USA | R | Gulka & Chang (1984b) |
| *Mytilus galloprovincialis* | Basque Coast Spain | C | Cajaraville & Angulo (1991) |
| *Ostrea edulis* (European flat oyster) | Atlantic Coast France | R | Comps et al. (1977b) |
| | | | Comps (1985a) |
| *Patinopecten yessoensis* (Japanese scallop) | Aomori Prefecture Japan | R | Elston (1986) |
| *Placopecten magellanicus* (Sea scallop) | Rhode Island USA | R | Gulka et al. (1983) |
| | | R | Gulka & Chang (1984a) |
| *Pecten maximus* (St. Jacques sea scallop) | Brittany France | R | Le Gall et al. (1988) |
| | | R | Le Gall et al. (1989) |
| | | R | Le Gall et al. (1991a) |
| | | R | Le Gall & Mialhe (1992) |
| | | R | Le Gall et al. (1992) |
| | Sweden, Scotland France | R | Le Gall et al. (1991b) |

**Table 3.** (cont.)

| Host Species | Geographic Region | R/C[1] | Reference |
|---|---|---|---|
| *Ruditapes philippinarum* | — | R | Comps (1983) |
| *Ruditapes decussatus* | Gulf of Tunis | C | Joly & Comps (1980) |
| | Portugal | R | Mialhe et al. (1987) |
| *Scrobicularia piperata* | Atlantic Coast France | C | Comps et al. (1979) |
| *Siliqua patula* (Pacific razor clam) | Washington state USA | R | Elston & Peacock (1984) |
| *Tapes japonica* (Japanese littleneck clam) | Washington state USA | R | Elston (1986) |
| *Tapes pullastra* | Brittany France | R or C | Auffret & Poder (1987) |
| *Tellina tenuis* | Scotland | R | Buchanan (1978) |
| *Tridacna crocea* (Burrowing clam) | Australia | R | Goggin & Lester (1990) |

[1]R = reported to be rickettsia-like.
C = reported to be chlamydia-like.

**Fig. 4.** Plaque containing rickettsia-like organisms in the digestive epithelium of the Japanese littleneck clam (*Tapes japonica*). Giemsa stain. Bar = 100 $\mu$m.

sible for these infections. They have been described as rickettsia-like or chlamydia-like (Table 3), but their taxonomy is not established. All are pleomorphic, chiefly coccoid to ovoid or rod-shaped, and giant forms, up to 9 $\mu$m in length, have been observed (Azevado and Villalba 1991). The ultrastructure of these organisms is similar to that described for the rickettsiae (Anderson et al. 1965), and many exhibit the pleomorphic development cycle typical of the order Chlamydiales or the genus *Rickettsiella*. Few have been tested for the group-specific chlamydial LPS antigen, but using the fluorescent antibody technique, both Meyers (1979) and Page and Cutlip (1982) demonstrated the presence of this antigen in the chlamydia-like organisms infecting the hard clam (*Mercenaria mercenaria*). Occasionally, virus particles are observed within these rickettsia-like and chlamydia-like agents (Harshbarger et al. 1977, Buchanan 1978, Meyers 1979).

Detection, description, and classification of the intracellular procaryotes infecting bivalves is limited to observations of the infected tissue using light and electron microscopy. None has been successfully characterized in vitro, and until this is done, understanding of these agents and the relationships among them will be incomplete. A lack of aquatic invertebrate cell lines has hampered efforts at in vitro propagation, although isolation has been attempted using a variety of means. By inoculation and incubation in hens' eggs, Buchanan (1978) isolated a rickettsia-like organism from *Tellina tenuis* but was unable to maintain it in subsequent passage. Gulka and Chang (1984a) used a homogenate of gill tissue from a diseased sea scallop (*Placopecten magellanicus*) to infect a homologous gill with a rickettsia-like agent in organ culture, but no secondary passage was attempted.

## CRUSTACEANS

Rickettsia-like and chlamydia-like organisms have also been reported in marine decapod crustaceans and in at least two species of freshwater amphipods (Table 4). Unlike the infections in bivalves, intracellular procaryotic infections in crustaceans frequently are associated with mortality which, at times, reaches epizootic levels (Federici et al. 1974, Leibovitz 1988, Krol et al. 1991).

In the freshwater amphipods, *Crangonyx floridanus* and *Rivulogammarus pulex*, rickettsia-like organisms undergo pleomorphic development cycles in intracytoplasmic vacuoles in host cells (Federici et al. 1974, Larsson 1982). The development stages range from small electron-dense rods, approximately 0.5 $\mu$m in length, to giant forms of up to 3 $\mu$m. In *C. floridanus*, paracrystaline arrays of the small electron-dense rods give infected individuals a pale green iridescent appearance. Federici et al. (1974) found infected cells concentrated in the epidermis and digestive tract, but vesicles containing the organisms were also free in the hemolymph. In *R. pulex*, Larsson (1982) found fat cells and connective tissue extensively infected, and other tissues were also involved. No iridescence was observed in these animals, but there was apparent crystal formation, similar to that described for *Rickettsiella popilliae* in insect hosts (Weiss and Moulder 1984). Morphological and developmental similarities of these agents to the insect pathogens of the genus, *Rickettsiella*, strongly suggest their placement in this genus, and Weiss and Moulder (1984) include the agent from *C. floridanus* in the species, *Rickettsiella grylli*.

The intracellular procaryotes infecting marine decapod crustaceans are not so readily classified. Those observed in penaeid shrimp are generally rod-shaped and range in length from ca. 0.7 to 1.6 $\mu$m. Although their ultrastructure has not been

**Table 4.** Rickettsia-like and Chlamydia-like Infections of Aquatic Crustaceans

| Host Species | Geographic Region | R/C[1] | Reference |
|---|---|---|---|
| *Crangonyx floridanus* (freshwater amphipod) | Florida USA | R | Federici et al. (1974) |
| *Rivulogammarus pulex* (freshwater amphipod) | Sweden | R | Larsson (1982) |
| *Cancer borealis* (Jonah crab) | Massachusetts USA | C | Leibovitz (1988) |
| *Cancer irroratus* (Atlantic rock crab) | Massachusetts USA | C | Leibovitz (1988) |
| *Cancer magister* (Dungeness crab) | Washington state USA | C | Sparks et al. (1985) |
| *Carcinus mediterraneus* (European shore crab) | France | R | Pappalardo & Bonami (1980) |
| *Paralithodes platypus* (Blue king crab) | Alaska USA | R | Johnson (1984) |
| *Penaeus japonicus* | — | C | Lightner et al. (1985) |
| *Penaeus marginatus* | Hawaii USA | R | Brock et al. (1986) |
| *Penaeus merguiensis* | Singapore Malaysia | R | Brock (1988) |
| *Penaeus monodon* | Singapore Malaysia | R<br>R | Anderson et al. (1987)<br>Brock (1988) |
| *Penaeus vannamei* | Hawaii/Texas USA | R<br>— | Krol et al. (1991)<br>Lightner et al. (1992) |

[1]R = reported to be rickettsia-like.
C = reported to be chlamydia-like.

extensively described, pleomorphic development stages are rarely reported. In contrast, the agents infecting crabs typically exhibit pleomorphic development stages similar to those of the Chlamydiae or the genus *Rickettsiella*. Most of the agents in crabs are rod-shaped and range in length from 0.2 $\mu$m to giant forms of 3.5 $\mu$m, but those associated with a highly lethal disease of laboratory-maintained Atlantic rock crab (*Cancer irroratus*) and Jonah crab (*Cancer borealis*) differ morphologically from the others. They are described as round to oval, ranging from 214 to 500 nm in the longest dimension (Leibovitz 1988).

The hepatopancreas typically is the target organ for the infections in marine decapods. Basophilic, Fuelgen-positive, intracytoplasmic inclusions or microcolonies, ca. 5-50 $\mu$m in diameter, are often noted in the epithelial or connective tissue cells of the hepatopancreas, and connective tissue throughout other organs is frequently involved. However, in the disease of Atlantic rock crab and Jonah crab, blood cells and hematopoietic tissues were primarily affected (Leibovitz 1988), and Sparks et al. (1985) observed a chlamydia-like infection that was systemic in the Dungeness crab (*Cancer magister*). Inflammation and aggregation of hemocytes are often noted in all of these diseases, and at times, there is extensive necrosis of host tissues (Sparks et al. 1985).

## DISCUSSION

Rickettsia-like and chlamydia-like infections are frequently reported in marine bivalves and aquatic crustaceans, and chlamydia-like agents are observed in numerous fish species. Until recently, few rickettsia-like agents were reported in fishes, but the increasing number of these reports in the last decade suggest fish diseases of rickettsial etiology may previously have gone unrecognized.

Only two of the many intracellular bacteria infecting aquatic poikilotherms have been sufficiently characterized for precise taxonomic placement. A new genus and species were established in the order Rickettsiales and the family Rickettsiaceae, for the salmonid fish pathogen, *P. salmonis* (Fryer et al. 1992), and the agent infecting the freshwater amphipod, *C. floridanus*, has been assigned to the insect-pathogenic species, *R. grylli* (Weiss and Moulder 1984). The remainder are considered to be rickettsia-like or chlamydia-like, based on morphology observed in the light and electron microscope.

More must be known of the biology of these organisms before they can be properly classified. They are morphologically diverse and range from pathogenic to benign in their respective aquatic hosts. Phenotypic similarities of morphology and development are apparent among many of those infecting each of the host groups, but advanced techniques, e.g. 16S rRNA analysis, will be required to determine the precise taxonomic placement of each and the relationships among them.

*Piscirickettsia salmonis* was described and characterized following isolation and propagation in cell culture. Understanding of the remainder of these agents will necessarily be limited until methods have been developed for their isolation and growth in the laboratory. At least two major challenges have restricted efforts at in vitro propagation. The most critical of these is a lack of appropriate culture systems. Although numerous cell lines have been developed from fishes and from insects, no cell lines originating from bivalves or decapod crustaceans are available for use, and axenic culture of rickettsial or chlamydial agents is rare.

Additionally, a high incidence of contaminating microorganisms provides a second major obstacle to culture. Many of the target tissues for the infections in aquatic animals, e.g. the gills, are exposed to the non-sterile aquatic environment. Selective systems, other than serial dilution, have not been developed to separate the rickettsia-like and chlamydia-like pathogens from more easily culturable microorganisms derived from the normal flora or the environment, and the potential sensitivity of the pathogenic agents to antibiotics and antimicrobials limits the usefulness of these compounds in the isolation procedures.

The epizootiology of the infections in aquatic poikilotherms is also difficult to study. Most available information concerning these diseases is from cultured populations of animals. However, infections are also observed in feral or non-cultivated hosts, and methods have not been developed for maintenance and culture of many of these host animals in the laboratory. In the absence of experimental animals, controlled in vivo studies of the disease process cannot be conducted. As techniques are developed for maintenance and/or culture of both the intracellular procaryotes and their aquatic animal hosts, a better understanding can be developed of the relationships among these agents and between them and previously-described rickettsial and chlamydial species.

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# 魚類、二枚貝及甲殼類之立克次體和披衣菌感染

John L. Fryer and Catharine N. Lannan

葛蘭氏陰性，胞內寄生的原核生物可感染許多水生變溫動物。一般為良性感染或致病性感染，這些原核生物偶具有極強病原性。雖然立克次體和披衣菌的分類地位尚未完全確立，但藉光學及電子顯微鏡觀察這些原核生物之形態及多變型發育期之有無，而常被描述為似立克次體或似披衣菌，目前僅有兩種被確定種名，只有一種被分離出來作體外定性。由於缺乏合適之培養系統，以及接種用的寄主組織內或表面常帶有污染源，以致分離和培養這些胞內病原體變得很困難。為了完全瞭解這些胞內原核生物，也為了確定這些原核生物彼此之間的關係，以及這些原核生物與已知的立克次體和披衣菌之間的關係，發展供實驗室培養並繼代這些似立克體和似披衣菌的技術，甚至於發展這種培養並繼代其水生寄主的技術都是刻不容緩的。

關鍵詞：立克次體, 披衣菌, 魚類, 二枚貝, 甲殼類。