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EFFECTS OF LOW TEMPERATURE ON THE DEVELOPMENT OF THE MICROSPORIDAN *Glugea stephani* IN ENGLISH SOLE *(Parophrys vetulus)*[1,2]

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Abstract: Glugea stephani requires temperatures above 15 C for development in juvenile pleuronectid flatfishes in Yaquina Bay, Oregon. The effect of low temperature (10 C) on the development of recently established parasites was tested experimentally in juvenile English sole *(Parophrys vetulus).* Low temperature arrested parasite development, but did not kill the protozoan which resumed development on return to 19-20 C after as long as 42 days at 10 C. No parasites detectable with the light microscope were found in fish examined after 70 days at 10 C. Although most juvenile English sole move permanently from the estuary to cooler ocean waters in the fall and do not contribute to the continuation of the parasite life cycle, the cycle may be maintained by low numbers of English sole that overwinter in the estuary.

INTRODUCTION

Adult English sole *(Parophrys vetulus)* rarely are found in estuaries on the Oregon Coast, but these areas are important as nursery grounds for young English sole which begin to arrive in the estuary as newly metamorphosed juveniles in January and emigrate to the ocean during the following fall.[4] Temperature requirements restrict the microsporidan protozoan *Glugea stephani* (Hagenmüller, 1899) Woodcock, 1904, to pleuronectid hosts that occupy the upper, warmer estuarine areas in Oregon. In English sole, the prevalence of infection with this parasite reaches levels above 50% in the upper Yaquina Bay estuary. Of those infected, 17% have been observed to carry infections of sufficient intensity to suggest a lethal outcome.[3]

Glugea stephani is dependent on temperatures above 15 C for development.[2,3] Juvenile English sole exposed to the parasite at 18 C and then held at 10 C do not develop infections detectable by light microscopy, but whether the parasite is killed or merely retarded by the 10 C temperature has not been determined. The purpose of this study was to experimentally examine the capacity of recently established *G. stephani* to survive a temperature below that allowing parasite development.

METHODS

Juvenile English sole were collected in the lower Yaquina Bay estuary during February, 1976, a time of year when *G. stephani* infected fish are never found.[3] These fish were held in tanks of running seawater and fed Oregon Moist Pellet[1] until their use in experiments in October, 1976. Spores for use in experiments were obtained from naturally-infected

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[2] Oregon State Agricultural Experiment Station Technical Paper No. 5723.

juvenile English sole collected in September, 1976 and were prepared and administered to experimental fish via brine shrimp *(Artemia salina)* as described by Olson.[3]

The experiment included six groups of fish, five groups exposed to *G. stephani* and one unexposed control group consisting of 6 fish. The unexposed controls were held at 19-20 C for the duration of the experiment (70 days). The other groups were exposed to *G. stephani* for 24 h at 18 C and then treated: one group of six exposed fish was held at 19-20 C for 70 days; three groups of 3 fish each were held at 10 C and then transferred to 19-20 C after 28, 35 or 42 days; and one group of 3 fish was held at 10 C for 70 days. All fish were held in tanks of static water changed daily.

At the termination of the experiment, intestinal tissue from all fish was examined for *G. stephani* cysts (xenomas) with the aid of a dissecting microscope. The tissue was fixed in Bouin's solution and then processed for histologic examination. The diameters of 25 representative parasite xenomas were measured from fish in each of the treatments that contained infected fish.

RESULTS

The results of the experiment are shown in Table 1. All fish exposed at 18 C and held at room temperature (19-20 C) developed massive infections and five of the six fish died before the experiment was terminated 70 days after exposure. All fish in the groups held at 10 C for 28, 35 and 42 days before return to room temperature developed macroscopic *G. stephani* infections. The period of time required for *G. stephani* to establish was about 24 h, but the absolute minimum time was not determined. The parasite xenomas developed over time at room temperature (Fig. 1 and Table 1). Fish held at 10 C for 70 days without return to room temperature did not develop infections detectable with the light

TABLE 1. Effects of 10 C temperature on the development of *Glugea stephani* after exposure to spores for 24 hours at 18 C.

Treatment	No. fish	Days at 10 C	Days at 19-20 C	Results	Mean xenoma diameter (μ m) (N = 25)	Standard deviation
A-Exposed Control	6	0	21-70[a]	All infected	Variable[c]	-
B-Unexposed Control	6	0	70	4 negative	-	-
				2 infected[b]	227.3	48.7
C	3	28	40-42	All infected	560.0	56.2
D	3	35	34-35	All infected	500.1	62.2
E	3	42	28	All infected	331.6	54.0
F	3	70	0	All negative	-	-

[a]English sole exposed and held at 19-20 C died with massive *G. stephani* infections after varying lengths of time.
[b]Unexposed control fish were held in two tanks, three fish in each. Two fish in one tank were lightly infected, possibly as a result of accidental exposure to spores.
[c]The size of xenomas varied in this group due to the great variation in survival time.

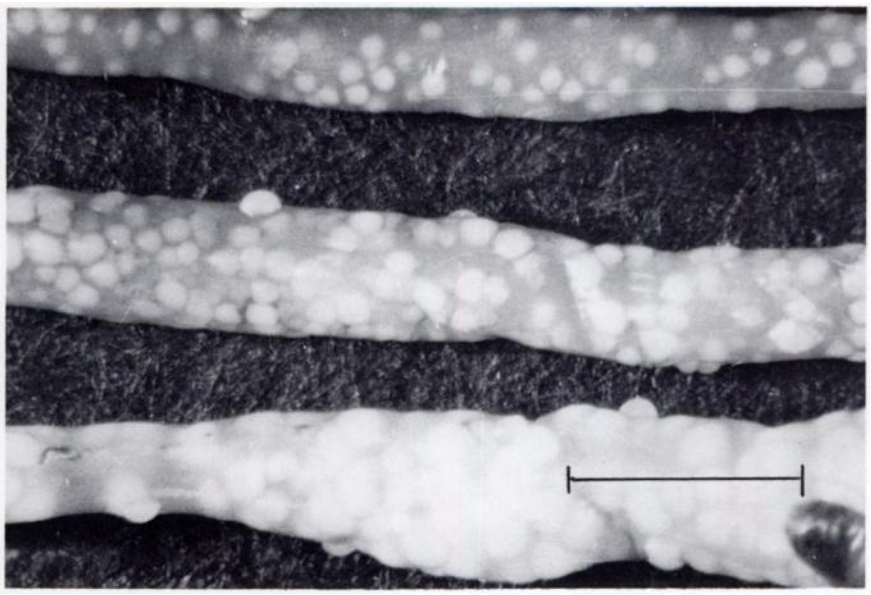

FIGURE 1. *Glugea stephani* infected intestines from juvenile English sole held at 10 C for 42 days, 19-20 C for 28 days (top); at 10 C for 35 days, 19-20 C for 35 days (middle); and at 10 C for 28 days, 19-20 C for 42 days (bottom). Scale = 5 mm.

microscope. Two of the six unexposed control fish were infected with small (~200 μm) *G. stephani* xenomas after 70 days at room temperature. These controls were in the same tank with an uninfected individual and may have been inadvertently exposed to spores which the uninfected fish did not ingest. The small size of the xenomas indicated that infection occurred after, not before, the experiment was begun. Three control fish in another tank were uninfected.

DISCUSSION

Temperature influences the development of *G. stephani* but 10 C does not kill the parasite once it is established. Under natural conditions, fish exposed to *G. stephani* at temperatures sufficient to allow the parasite to become established will not lose the infection by moving to areas of cooler water temperatures for return to warmer water would permit resumption of parasite growth. In fish that become infected shortly before emigrating from the estuary to the ocean, the parasite probably never becomes manifest.

English sole spending substantial amounts of time in the upper Yaquina Bay estuary, where warm water temperatures (15 C) allow *G. stephani* infection and development, are subject to potentially lethal infections. Yet those that survive and undertake the fall movement to the lower estuary and thence to the ocean do not contribute to the spread of the parasite, for they never again occupy an environment favorable for the parasite. The success of a parasite with temperature requirements that restrict it to very small geographic areas on the Oregon Coast is unexpected. *Glugea stephani* must kill some portion of the host population in warm water areas

since no other mechanism for spore release has been observed.[3] Severe and, therefore, potentially lethal infections in juvenile English sole are most prevalent from September through November. Release of spores by dead and decomposing English sole in the upper estuary likely occurs during these months. While water temperatures at this time may allow infections to become established, temperatures soon become less than optimal for parasite development.[3] The majority of the juvenile English sole leave the estuary during this period, but a few remain through the winter months and into the following summer.[4] Overwintering fish could carry latent infections and serve as a source of spores for infection the next year. Other possible sources include spores overwintering in sediments, in crustacean vectors or in infected juvenile starry flounders *(Platichthys stellatus)*. Spores in sediments would require accidental ingestion and would be an inefficient mechanism. Current evidence indicates that spores pass directly through the crustacean digestive tract.[3] Young-of-the-year starry flounders are infected with *G. stephani*, but these fish are present in such low numbers in Yaquina Bay (Olson, unpubl.) that they probably do not contribute substantially to maintenance of the parasite cycle.

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