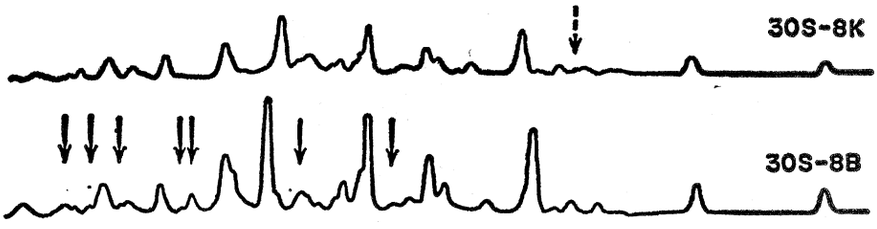

Fig. 1. Chromatography (Dowex 50) of the tryptic peptides of proteins 30*S*-8B and 30*S*-8K (*4*). The arrows mark peaks which occur in one pattern and not in the other.

The relatively greater content of arginine and lysine in 30*S*-8B is also reflected in the tryptic peptide pattern of this protein, which is compared to that of 30*S*-8K in Fig. 1. There are several more tryptic peptides present in 30*S*-8B than there are in 30*S*-8K. We have also observed a few minor peptides in 30*S*-8K which are different from those in 30*S*-8B. Finally, the tryptic peptides of a different protein, 30*S*-9, have been analyzed with samples obtained from B and K strains. These two homologous proteins are indistinguishable.

If it is assumed that the four methionines of 30*S*-8 are all internal amino acids, then cyanogen bromide digestion of both 30*S*-8B and 30*S*-8K should yield five major peptides, with one or more of those from 30*S*-8B more basic than the homolog of 30*S*-8K. This prediction has been verified.

While four of the cyanogen bromide fragments from these two proteins are indistinguishable, one of them is significantly different in the two proteins. This observation suggests that the amino acid replacements responsible for the different charges of the two homologous proteins are restricted to one region of protein 30*S*-8.

All the data indicate that proteins 30*S*-8B and 30*S*-8K have different amino acids at several positions in their primary sequences. Therefore, the K locus is the first positively identified genetic determinant for the primary structure of a ribosomal protein.

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Vibrio parahaemolyticus from the Blue Crab Callinectes sapidus in Chesapeake Bay

Abstract. *Strains of* Vibrio parahaemolyticus, *the etiologic agent of "Shirasu" food poisoning in Japan, were isolated from moribund blue crabs* Callinectes sapidus *and identified by biochemical and serological techniques.*

Since 1950, when Fujino *et al.* (*1*) isolated a new species of bacteria during an outbreak of "Shirasu" food poisoning, a series of papers has appeared describing the taxonomy and epidemiology of the etiologic agent *Vibrio parahaemolyticus* (*2*). The first recorded isolations of *V. parahaemolyticus* in the United States reported this species in seawater, sediments, and shellfish from the Puget Sound region (*3*). From estuarine sediments collected from the Gulf of Mexico and southern Atlantic coasts, Ward (*4*) isolated organisms related serologically to *V. parahaemolyticus*.

During studies of mortality in populations of the blue crab *Callinectes sapidus*, strains of bacteria *Vibrio parahaemolyticus* were isolated from lethargic and moribund crabs being retained in commercial tanks during "shedding" of soft crabs. Mortality of crabs in some tanks was in excess of 50 percent. Dead animals did not have the signs or the etiological agent associated with the "gray crab" disease (*5*). Animals from which bacteriological samples were taken were abnormally weak, and examination of their hemolymph revealed large numbers of bacteria. Broken claws and appendages consistently contained necrotic, liquefied tissue, and large numbers of bacteria were found in smears examined with the phase microscope.

Inoculation of brain-heart infusion agar (Difco, NaCl added to adjust final salt concentration to 1.5 percent) with material from necrotic claws and with hemolymph removed aseptically from 16 animals permitted isolation of a number of strains of bacteria. Of 28 pure cultures selected from the most abundant colony types that appeared on the brain-heart infusion agar, 21 cultures were identified as *Vibrio parahaemolyticus*.

Identification procedures included taxonomic analysis by computer with 210 coded features. The 21 *V. parahaemolyticus* strains clustered at *S* equal to 82 percent with known strains (*6*). This degree of similarity is well above that usually accepted for species (*7*).

The base composition of the DNA of *V. parahaemolyticus* isolates from blue crabs ranged from 44 to 46 mole percent of guanine plus cytosine. Base compositions were determined by melting temperature measurements of purified DNA (*8*). This range is well within that determined for *V. parahaemolyticus* in a previous study (*9*). Serological confirmation of the diagnosis was made by slide agglutination tests with antiserums prepared against K antigens (*10*).

Strains of *V. parahaemolyticus* isolated from diseased blue crabs demonstrated lipase and lecithinase activity and were capable of liquefying gelatin and hydrolyzing casein. These properties may contribute to the invasiveness of the bacteria.

Previous reports on *V. parahaemolyticus* described its involvement in outbreaks of food poisoning in humans. Epidemiologic studies of "Shirasu" in Japan have suggested the existence of a nonhuman reservoir of *V. parahaemolyticus*, and Akazawa (*11*) reports frequent isolation of this bacterium from diseased marine and estuarine fishes.

This is the first isolation of *Vibrio parahaemolyticus* from diseased crabs and from the Chesapeake Bay region. *Vibrio parahaemolyticus* is very likely

part of the marine flora, and occasionally it invades marine animals where it may become a potential human health problem.

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Larval Trematodes: Double Infections in Common Mud-Flat Snail

Abstract. *Larvae of the trematode* Zoogonus lasius *are involved in most double infections of* Nassarius obsoleta. *The two most common trematode parasites of this snail do not occur together in double infections. Double infections were found in 14 of 340 infected snails in a total sample of 5025 snails.*

There have been a number of studies on snails infected with more than one species of larval trematode. Cort *et al.* (*1*) demonstrated that while some species of trematode larvae coexisted in the same host, other combinations of species were rarely or never found together. Ewers (*2*) suggested that infection with one species of trematode predisposes some mollusks to infection with another species. Recent evidence supports the idea that echinostome redia are antagonistic to development of another species of larval trematode in the same host (*3*). With some species, echinostome larvae can completely eliminate a competing species, while in other instances the echinostome remains dominant but does not completely eliminate the other species.

The common mud-flat snail *Nassarius obsoleta* has been recorded as the first intermediate host for at least eight species of larval trematodes in the area of Beaufort, North Carolina. Some of the snails serve as a host for double infections. This study was undertaken to determine the extent of such double infections in a natural population and what combinations of larvae could be found coexisting in a host.

Nassarius obsoleta were collected between 17 June and 25 July 1968. The snails were isolated in finger bowls and checked for shedding cercariae. Snails which were shedding cercariae were then dissected and their tissues examined. Of 5025 snails, 340 (or 6.8 percent) were infected, 326 of them with a single trematode, 14 with double infections (Table 1). In half of the double infections, both species of cercariae were being shed. The others were shedding but one species, and the double infection was obvious only upon examination of the digestive gland of the snail. A comparison of the actual number of double infections with the expected number (assuming that the proportion of double-infected snails among the total infected snails was equal to the proportion of infected snails among the total population, and that all double-infected snails can be detected) gave a chi-square value of 2.41, which is not significant. Therefore, out of this population sample, the number of double infections did not differ significantly with the expected number.

Table 1. The most commonly found species of larval trematodes as found in 5025 *Nassarius obsoleta*. There were 326 single infections, and 14 double infections.

Species in single infections	Single infections (No.)	Double infections (No.)
Lepocreadium setiferoides	103	5
Himasthla quissetensis	89	3
Zoogonus lasius	72	12
Strigeid cercaria (probably *Cardiocephalus brandesii*)	27	1
Austrobilharzia variglandis	20	3
Stephanostomum dentatum	14	2
Gynaecotyla adunca	1	0
Monostome cercaria	0	2

The combinations of infections that did occur were not significantly different than would be expected on the basis of relative abundance of species found in single infections. There are, however, two points that are statistically significant. (i) One species, *Zoogonus lasius*, is involved in 12 of 14 double infections. When the actual number of *Z. lasius* involved in double infections is compared with the expected number, the chi-square value is 9.2 with a probability of .005. Thus it is highly improbable that this many larvae of *Z. lasius* would be involved in a double infection based on random selection. (ii) The most common trematodes, *Lepocreadium setiferoides* and *Himasthla quissetensis*, were never found together (chi square, 6.6; $P < .01$). Again, it is quite unlikely that these two would not be found together if the double infections were the result of random selection.

It is not clear from the present study why *Z. lasius* is involved in such a high percentage of the dual infections. This species is not the most commonly found larval trematode in *N. obsoleta*.

It has been demonstrated that each of the larval trematodes alters the thermal acclimation patterns of cytochrome c ovidase in the host tissue. Furthermore the alteration in host response is distinctive for each species of trematode. That is, the thermal acclimation patterns of this enzyme in digestive gland tissue from snails infected with one species of trematode is quite different from that of the same tissue from snails infected with another species (*4*).

Since the larvae are carefully dissected out of the host tissue before the assays are made, these differences in response are not due to the parasite itself, but rather to the alterations in the host tissue due to the presence of the parasite. This suggests that