by *N. canicruria* arg-2 str-s, prototrophs have not been detected. Is close linkage responsible for failure to find recombination? Significantly, crosses of *N. canicruria* arg-2 with *N. erythropolis* mutants do not produce prototrophic recombinations, whereas *N. erythropolis* mutants crossed with *N. canicruria* ade-2 are generally fruitful (Table 1). Such evidence would seem to indicate that, if linkage is a factor, the *N. canicruria* arg-2 locus should be more closely linked with those loci thus far discovered in mutants of *N. erythropolis*. Thus, the *N. canicruria* arg-2 locus must not be closely linked with the *N. canicruria* ade-2 locus; accordingly, linkage is probably not responsible for undetected recombinational events in *N. canicruria* by *N. canicruria* crosses.

Our experiments seem to warrant the following conclusions. Crosses of *N. canicruria* or *N. erythropolis* mutants of homologous origin are not fertile or at least are not detectable by means of the procedures used in our experiments. Crosses of mutants of heterologous origin, *N. erythropolis* by *N. canicruria*, are fertile. Thus nocardial recombination seems to be governed by a compatibility system. Compatibility systems have been reported for both the streptomycetes (*6*) and *Escherichia coli* (*7*); however, nocardial compatibility differs in one respect. At least one member of a pair capable of genetic interaction in the *E. coli* or streptomycete systems is compatible with mutants of homologous origin (*6, 7*), but this has not been so in the nocardial system studied.

In the case of *E. coli* an infectious F agent is responsible for the compatibility (*8*). The possibility that an infectious F-like agent exists in the nocardial system was tested in the following manner. *Nocardia canicruria* ade-2 was grown in mixed culture with *N. erythropolis* ade-1 his-3 for 3 days. Several individual strains of the *N. canicruria* and *N. erythropolis* mutants were re-isolated and tested to ascertain nutritional requirements and to affirm their identities. These strains were then tested with complementary auxotrophs of the homologous species. All such mixtures failed to result in detectable recombinational events. Hence, in contrast to the system in *E. coli* controlling compatibility, our data indicate that mating types governing nocardial recombinational events are independent of an infectious F-like factor.

These recombinational events in bacteria are the first evidence suggesting heterothallism similar to that observed in the true fungi (*9*). Because the crosses *N. erythropolis* ade-1 his-3 str-s by *N. canicruria* ade-2 str-r and *N. erythropolis* ade-1 his-3 str-r by *N. canicruria* ade-2 str-s gave recombinant prototrophs having the streptomycin phenotype of the *N. canicruria* strain used, *N. erythropolis* could be regarded as the male and *N. canicruria* as the female. Preliminary data indicate that the frequency of recombination may be as high as 1×10^{-5} parent cells and that the recombinants do not readily segregate (*10*).

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References and Notes

1. J. N. Adams and N. M. McClung, *J. Bacteriol.* **84**, 206 (1962); R. S. Breed, E. G. D. Murray, N. R. Smith, *Bergey's Manual of Determinative Bacteriology* (Williams and Wilkins, Baltimore, ed. 7, 1957), p. 694.
2. R. Lieske, *Morphologie und Biologie der Strahlenpilze* (Borntraeger, Leipzig, 1921); G. H. G. Davis and J. H. Freer, *J. Gen. Microbiol.* **23**, 163 (1960).
3. J. Lederberg, *Science* **122**, 920 (1955); F. Jacob and E. Wollman, *Sexuality and the Genetics of Bacteria* (Academic Press, New York, 1961).
4. S. G. Bradley, *Ann. Rev. Microbiol.* **16**, 35 (1962); D. A. Hopwood and G. Sermonti, *Adv. Genetics* **11**, 273 (1962).
5. J. Lederberg and E. M. Lederberg, *J. Bacteriol.* **63**, 399 (1952).
6. S. G. Bradley and D. L. Anderson, *Proc. Soc. Exptl. Biol. Med.* **99**, 476 (1958); *J. Gen. Microbiol.* **23**, 231 (1960).
7. J. Lederberg, L. Cavalli, E. M. Lederberg, *Genetics* **37**, 720 (1952); P. Mäkelä, J. Lederberg, E. M. Lederberg, *ibid.* **47**, 1427 (1962).
8. A. J. Clark and E. A. Adelberg, *Ann. Rev. Microbiol.* **16**, 289 (1962).
9. C. Alexopoulos, *Introductory Mycology* (Wiley, New York, 1962).
10. Supported in part by grant E-4063 from the National Institutes of Health. We thank Mary D. Armknecht for technical assistance.
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Calorific Values of Microcrustacea

Abstract. *The heat content of copepods and other microcrustaceans and two species of algae in calories per gram varied from 4427 for immature crayfish to 5643 for the female* Diaptomus siciloides. *The algae, cladoceran, anostracan, conchostracan, and immature crayfish were all below 5000, whereas all the copepods were above 5300 ($\bar{x}$ 5467); thus copepods may contribute more energy to the food chain on a weight basis than other lower forms of crustacea.*

Studies of energy transformation have pointed out the need for determining the amount of heat that various organisms from a wide taxonomic range and a large variety of environments can produce (*1–3*). Some data are available for the plankton, but very few are available for copepods, either marine or fresh-water. We have studied calanoid copepods, a cyclopoid, cladocerans, and several other organisms.

Our interest in calorimetry has centered around energy transformations in populations of *Diaptomus siciloides* (*4*), *Mesocyclops edax*, and *Diaptomus leptopus*. The pertinent data in the present paper have been used in the computation of the energy budget for *D. siciloides* (*4*).

All determinations were made with a Parr model 1411 oxygen bomb calorimeter (*5*) and the standard Parr method, with modifications for small samples, was used. Millipore membrane (pore size, 5 μ) was burned with each sample as filler material. The filter membrane was first compressed into pellet form with a punch and die from a Parr pellet press (*6*) (except for the 5-μ disk onto which the sample was filtered). Twenty determinations on this pellet alone yielded a mean calorific value of 3104.6 $\pm$37.0 cal/g (coefficient variation of 1.19). Ten determinations on 0.45-μ membrane yielded a mean value of 2935.2 cal/g. To find the variation due to sample size, samples of this membrane varying from 6 to 95 mg were burned. A plot of the absolute variation from the mean against the sample size showed that the variation increased rapidly as the sample got smaller. Samples larger than 30 mg approached the mean with an accuracy of $\pm$ 7 to 17 cal/g. The membrane pellet burned usually weighed more than 30 mg, and the pellet and organisms together usually weighed more than 50 mg—in the 70-mg range most frequently. Determinations were made on an electrobalance.

Some of the animals from a sample were segregated onto a membrane which was then dried for 24 hours at 60°C, and other specimens were measured to determine their mean length and width. The weight of the animals determined the number used; we could maintain

sample weights at not less than 5 mg, but most frequently the weight was near 10 mg and many samples weighed more.

All determinations reported are ash-free; corrections were made for ash when it was present. For the copepod and cladoceran determinations, there was no ash, for the *Caenestheriella setosa,* the unidentified conchostracans, and the alga, *Anacystis*, there were varying amounts. However, for the latter the correction was insignificant and the data are unmodified. When ash was obtained it was not incinerated further to determine its combustibility.

The number of calories per gram dry weight for the fresh-water organisms varied from 4427 for the immature crayfish to 5643 for the female *Diaptomus siciloides.* The data on *Diaptomus arcticus* eggs are from two determinations of 100 egg sacs each ($\bar{x}$ = 72 eggs per sac) and are about 2.6 percent higher than the data for the females from which they were taken. This indicates that eggs have approximately the same calorific value as the adult females for this species. All the species of *Diaptomus* are very similar even though they range in size from 0.75 to 2.80 mm. Females are slightly longer than males (about 1.8 percent).

The lowest values were from *Daphnia pulex* and the immature crayfish, neither of which left any ash. No explanation is advanced for these lower values. It is doubtful that on a weight basis starvation would make any significant difference. Richman's data (*1*) on *Daphnia*, in which the state of nutrition was known, indicate that values for cultured *Daphnia* are 4059, 4124, and 5075 for animals measuring 0.7, 1.3 and 1.8 mm, respectively. Since our *Daphnia* measured 1.92 mm, they would be most comparable to Richman's larger animals. Perhaps *Daphnia* in nature do not have as high a calorific value as cultured ones.

Daphnia schødleri were also burned, but their values were not included in Table 1 because they were preserved animals, and this might have contributed to the low value obtained, 4361 ± 213 cal/g (coefficient of variation 4.89).

Conchostracans (*Caenestheriella setosa*) taken in 1961 were from 4 to 7 mm long and, on burning, left a large ash deposit which averaged 19.4 percent of total dry body weight. Determinations made in 1962 on smaller (1.5 to 3 mm) unidentified conchostracans were ash-free. On the assumption that these are the same species, and this is highly probable because they were taken from ponds less than 10 miles apart, it is suggested that calcium deposition in the shell of these animals is made sometime after they reach 3 mm in length. Calorific determinations may possibly yield valuable information about shell deposition in small mollusks.

Table 1. Calorific values of fresh-water microcrustacea and two species of algae. Length measurements are of the cephalothorax only. Total length is, approximately, 1.3 times the length of the cephalothorax. Abbreviations: S.D., standard deviation; C.V., coefficient of variation. The measurement given for eggs is the diameter.

Species	Sex	Date collected (1962)	Total burned		Length (mm)	Cal/g dry wt.	S.D.	C.V.
			No.	Wt. (mg)				
Diaptomus arcticus	♂	18 June	500	58.32	2.34	5468	342	6.24
	♀	18 June	500	64.06	2.80	5526	277	5.02
D. arcticus egg sacs		18 June	200	10.97	0.15	5672		
D. leptopus	♂	25 June	3000	91.84	1.45	5396	387	7.16
	♀	25 June	2100	56.99	1.70	5436	214	3.93
D. siciloides	♂	16 July	8500	33.09	0.75	5334	242	4.53
	♀	16 July	12500	61.30	0.95	5643	75	1.32
Mesocyclops edax	♀	16 July	4700	48.37	0.85	5478	97	1.78
Daphnia pulex		11 June	2000	72.31	1.92	4478	372	8.31
Streptocephalus seali		10 Aug.	8	243.77	10–15	4932	184	3.72
Crayfish, immature		9 June*	5	42.44	10–20	4427	370	8.29
Caenestheriella setosa		9 June*	39	187.26	4–7	4560	270	5.93
Conchostracan, undetermined		11 June	229	148.20	2–3	5205	116	2.23
Anacystis sp.		3 Aug.*		68.31		4781	812	16.99
Pandorina morum, culture		July		42.88		4969		

* 1961

Measurements are given to show that for the copepods the shorter animals provide the same number of calories per gram dry weight as the longer animals. For the cladoceran, anostracan, and conchostracans the calorific values are lower even though their size is relatively large.

Data on *Calanus finmarchicus* specimens taken monthly from April 1962 through March 1963 at Millport, Scotland emphasize some interesting differences between those freshwater *Diaptomus* listed and the marine copepod, *Calanus.* With few exceptions the values for *Calanus* in calories per gram dry weight were substantially higher than those of the three species of *Diaptomus* (♂ $\bar{x}$ = 5400, ♀ $\bar{x}$ = 5535). Also a seasonal cyclical fluctuation in the calorific values for *Calanus* was clearly apparent. The *Calanus* females ranged from 5232 in August to 6626 in October (entire year, $\bar{x}$ = 5914). All values above 6000 occurred with animals collected from October through February. The number of calories per female ranged from 0.62 in August to 1.6 in December and January (entire year, $\bar{x}$ = 1.2). For the males the range was 5334 in November to 7203 in December (entire year, $\bar{x}$ = 6475); values were above 6500 from April through August and again in December (7203), January (6859), and February (6871). Also in December and January calories per animal were highest, 1.9 and 1.8 (entire year, $\bar{x}$ = 1.4). Values for the stage V (final larval stage) of copepodites were higher than those for the adult males and females. The two lowest values obtained were 6637 in April and 7050 in May. All the other determinations were above 7387 (August), and the average for the entire year was 7416; range, 6637 to 7672. The October-through-February values as calories per gram were consistently above 7500. There were no March copepodite determinations. The mean value as calories per animal was 1.7; range, 1.4 in April to 2.17 in December. Since the stage V copepodites are the overwintering form and have a large amount of fat stored in their body, their higher calorific value is expected.

When calorific data are expressed in terms of weight, there is no apparent relation between the length (as shown for *Diaptomus*) and calorific value per gram dry weight in *Calanus.* Males measured 2.24 to 2.52 mm, but the calorific values for the 2.5 mm males ranged from the lowest (5334) to the highest (7203). Females ranged from 2.24 to 2.72 mm, while the highest calorific values were obtained for females between 2.41 and 2.55 mm. A similar lack was shown for the copepodites, ranging in length from 2.09 to 2.39 mm. The highest calorific values were obtained from animals of intermediate size, approximately 2.25 mm.

The data of Golley (*3*), Richman (*1*), Slobodkin and Richman (*7*), and Richman and Slobodkin (*8*) are extended and supported by our data (*9*).

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References and Notes

1. S. Richman, *Ecol. Monographs* **28**, 273 (1958).
2. F. Golley, "Table of Calorific Equivalents" (Univ. of Georgia, Athens, 1958), mimeographed.
3. F. Golley, *Ecology* **42**, 581 (1961).
4. G. W. Comita, *Proc. Intern. Assoc. Theoret. Appl. Limnol.*, in press.
5. For description of oxygen bomb calorimeter see Parr manual No. 128 (1958).
6. For description of pellet press, see Parr manual No. 130 (1960).
7. L. Slobodkin and S. Richman, *Nature* **191**, 299 (1961).
8. S. Richman and L. Slobodkin, *Bull. Ecol. Soc. Am.* **41**, No. 3, 88 (1960).

9. We thank Dr. J. W. Constantine, R. J. Schindler, and Sandra Werre for their help in the early phases of this study; Dr. Ralph W. Dexter for the identification of *Caenestheriella setosa;* and Dr. S. M. Marshall and Dr. A. P. Orr for the *Calanus* specimens and for permission to publish their calorific values. Supported by the National Science Foundation grant 16415 and the North Dakota Institute for Regional Studies.

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Electrocardiographic Studies of Free-Swimming Sharks

Abstract. *Electrocardiograms were taken of young lemon sharks,* Negaprion brevirostris, *as they swam freely in a circular concrete pool. Electrodes attached to the fin and torso yielded negligible or minute deflections, but direct leads yielded satisfactory recordings.*

Few accounts of the elasmobranch electrocardiogram are available: Kisch (*1*) has reported epicardial and endocardial tracings from two species of sharks and two species of skates which were anesthetized and submerged in a small tank; Satchell (*2*) studied restrained dogfish. However, up to the present, no one has taken an electrocardiogram of a free-swimming marine animal although King *et al.* (*3*) with difficulty recorded a few beats from the heart of a beluga whale. We have devised a method for the electrocardiography of freely moving sharks in a pool of flowing sea water.

In preliminary experiments we found that electrodes attached to the fin and torso yielded minimal deflections because the shark's heart is so well insulated by its cartilaginous skeleton. Hence direct leads were essential, but they present problems because of the proximity of the heart to the cartilage. Extracardiac structures in the shark have high electrical resistance not found in electrocardiography of the higher vertebrates.

A free-swimming lemon shark (*Negaprion brevirostris*), approximately 1 m long, was anesthetized (*4*) with MS-222 (tricaine-methane-sulfonate), removed from the water, and strapped to a restraining board (*5*). The gills were perfused with fresh sea water. We used an atraumatic electrode with a 20-gauge hypodermic needle in a plastic jacket that extended 2 mm beyond the tip of the needle. The lumen of the plastic tube, distal to the needle, was filled with agar saturated with sea water. A single exploring electrode was placed over the ventricular epicardium through a drill hole in the skin and coracoid cartilage in the midventral line. The flexible conducting cable (Alpha wire No. 26), 3 ft long, was sutured to the skin at two points as it passed around the right side of the shark to the base of the first dorsal fin where it was again securely sutured. The distal end of the cable with an insulated waterproof connector trailed freely from the leading edge of the fin. The shark was then returned to a circular concrete pool, 4 m in diameter and 40 cm deep, where it recovered rapidly. Two hours later the first tracings were recorded. The shark's electrode cable was attached to a longer cable which passed to a swivel 3 m above the center of the pool and thence to a Sanborn Viso-Cardiette at the side of the pool. The indifferent electrode and ground wires were placed in the water near the edge of the pool (*6*). Electrocardiograms were recorded at paper speeds of 25 and 50 mm/sec with standardization recorded for each experiment.

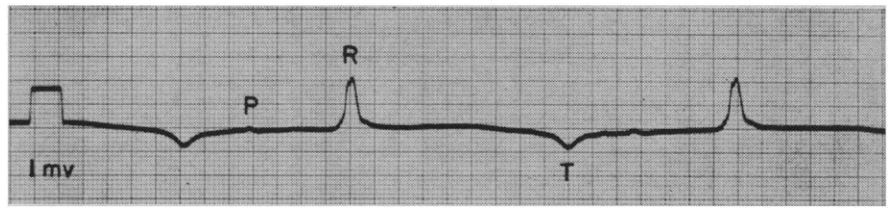

Fig. 1. Representative electrocardiogram of a free-swimming lemon shark (paper speed, 50 mm/sec).

Over a period of 3 years we obtained more than 200 electrocardiographic recordings from 12 lemon sharks about 1 m long and weighing approximately 8 kg. Each shark was studied repeatedly on two or more successive days. A representative electrocardiogram is presented in Fig. 1.

The records made while the shark was resting at the bottom of the pool showed clearly defined waves synchronous with movement of the gills, with a frequency of about half the cardiac rate. However, during active swimming, gill movements were absent since water passed continuously through the open mouth and out the gill slits. Under these circumstances the electrocardiographic deflections appeared with a stable rather than an undulating base line.

The cardiac rate ranged between 30 and 65 beats per minute as the temperature of the water varied. The rate was relatively fixed for a given shark and did not change significantly with various stimuli: tapping the side of the pool, waving the hand, splashing water, or placing dye in the water.

Average measurements in seconds are: P waves, 0.06; P-R interval, 0.38; QRS complex, 0.09; and Q-T interval, 1.04. The ventricular depolarization and repolarization deflections are clearly defined. No attempt is made to analyze magnitude and direction of the deflections because the relationship between the exploring electrode and the heart was not fixed.

We believe that our technique facilitates the study of cardiac function in elasmobranchs and other marine vertebrates under conditions approaching more closely those found in nature than when the animal is restrained, anesthetized, or removed from the water (*7*).

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References and Notes

1. B. Kisch, *Exptl. Med. and Surg.* **6**, 31 (1948); **7**, 55 (1949).
2. G. H. Satchell, *J. Exptl. Biol.* **37**, 719 (1960).
3. R. L. King, J. L. Jenks, P. D. White, *Circulation* **8**, 387 (1953).
4. P. W. Gilbert and F. G. Wood, *Science* **126**, 212 (1957).
5. P. W. Gilbert and S. D. Douglas, *Anat. Record* **138**, 351 (1960).
6. F. N. Wilson, F. D. Johnston, F. Rosenbaum, P. S. Barker, *Am. Heart J.* **32**, 277 (1946).

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