Glucose turnover in kelp bass (*Paralabrax* sp.): in vivo studies with [6-3H,6-14C]glucose

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BEVER, KAREN, MAYMIE CHENOWETH, AND ARNOLD DUNN. Glucose turnover in kelp bass (*Paralabrax* sp.): in vivo studies with [6-3H,6-14C]glucose. Am. J. Physiol. 232(1): R66-R72, 1977 or Am. J. Physiol.: Regulatory Integrative Comp. Physiol. 1(1): R66-R72, 1977. — [6-3H,6-14C]glucose was injected via an indwelling arterial cannula in free-swimming, fed, and fasted kelp bass to determine hepatic glucose production, peripheral glucose uptake, minimal glucose mass, mean transit time, and the percent of carbon recycling under the two different nutritional states. Mean plasma glucose levels remained unchanged in fed and fasted fish (48 ± 8 vs. 43 ± 8 mg/100 ml). During steady-state conditions, glucose replacement rates of fed and fasted fish determined with [6-3H]glucose are similar (0.035 ± 0.006 vs. 0.025 ± 0.003 mg/min per 100 g) and do not differ from rates determined with [6-14C]glucose (0.035 ± 0.005 vs. 0.026 ± 0.002). The minimal glucose masses and the mean transit times determined with both isotopes are also similar suggesting that plasma glucose levels and glucose turnover are maintained in fish fasted up to 40 days with no apparent increase in carbon recycling. Nonsteady-state isotope experiments suggest that these fish can alter rates of hepatic glucose production and peripheral uptake in response to hyper- and hypoglycemia.

PAST INVESTIGATION of glucose metabolism and its regulation in intact fish have relied primarily on the measurement of net changes in circulating glucose levels in animals challenged with prolonged starvation or injected with glucoregulatory hormones (2, 3, 11, 19, 22). Reported results are often contradictory and complicated by inter- and intraspecies variability. Isotope studies of metabolism in free-swimming fish have been limited by the inherent problems involved in both administering isotope into and sampling from the circulation of these organisms. Both Brown (1) and Hochachka and Ikeda (18) using [6-14C]glucose. After killing the carp, these investigators also measured the randomization of [14C] from carbon 6 into carbons 1-5 and suggested that gluconeogenesis takes place. With the exception of one report by Tashima and Cahill (21) no attempt has been made to measure glucose turnover, production, or uptake by isotopic means in intact fish and these results were not quantitative.

In the present study, isotope experiments were performed in kelp bass (*Paralabrax clathratus*) having an indwelling arterial cannula which allowed intravenous administration of the isotopes and serial sampling of the circulation. [6-3H,6-14C]glucose was used to measure hepatic glucose production, glucose uptake by the periphery, the minimal glucose mass, and the amount of carbon recycling according to the procedures of Katz et al. (12).

METHODS

Maintenance of experimental animals. Kelp bass, *P. clathratus* (150-500 g), were obtained by trapping in Big Fisherman's Cove, Catalina Island, and maintained in 50-gal aquariums with fresh, running seawater at ambient ocean temperatures (18-22°C). Thawed squid were provided daily as a readily acceptable food source. Resumption of feeding after capture was the criterion used to indicate adaptation to the new environment of the aquarium. A fasting state was obtained by withholding food for at least 20 days; in some animals, food was withheld for as long as 42 days. These prolonged periods are required to establish a fasting state in these fish since examination of gut contents indicated that approximately 2 wk are required for complete passage of food through the digestive tract (personal observations).

Cannulation of the ventral aorta. Anesthesia is induced with tricaine methanesulfonate (Aldrich) in seawater (1:10,000) and maintained by perfusing water containing the anesthetic (1:10,000) across the gills during surgery. The bulbus arteriosus and ventral aorta are exposed by a small incision in the membrane lining the gill chamber just over the heart and any interfering muscle tissue is carefully retracted. The cannula consists of 70 cm of polyethylene tubing (PE-60), attached to the shank of a 21-gauge hypodermic needle which has a bend of 120 degrees. This cannula, filled with heparinized Courtland saline (100 U/ml) prepared without glucose (24), is inserted into the ventral aorta anterior to the bulbus arteriosus and posterior to the branching of the first gill artery (Fig. 1). Since fish blood vessels are considerably less durable and elastic than those of mammals, extreme care must be exercised in all procedures. When the cannula is in place, blood pulses back into the tubing. Fresh heparinized saline is used to clear blood...
from the cannula and the free end is heat sealed. The cannula is then anchored firmly to the skin with silk sutures so that the tubing causes minimal interference with opercular and pectoral fin movements. Recovery from anesthesia is effected by perfusing the gills with fresh seawater. The fish is then placed in an experimental tank for the duration of the experiment. The entire procedure takes no longer than 25 min. The animals are not used for 3–4 days after the surgery. The cannula is flushed daily with heparinized saline to prevent clotting.

Experimental protocol. Tracer quantities of [6-3H, 6-14C]glucose (1 μCi14C, 2 μCi3H) (New England Nuclear) are injected via the indwelling cannula at time 0. The cannula is then repeatedly flushed with saline to prevent isotope contamination of early blood samples. Serial blood samples (0.10–0.15 ml) are drawn at appropriate intervals after isotope administration. The total amount of blood taken during the experiment is always less than 10% of the estimated blood volume. Every attempt was made to avoid any disturbance which would cause rapid swimming by the experimental animal.

Chemical analysis and determination of radioactivity. The plasma glucose was assayed with the glucose oxidase procedures of Meites and Bohmann (17). The glucose specific activity was determined as previously described (6).

Calculations. The steady-state plasma glucose replacement rate determined from [6-3H]glucose (Rg) and [6-14C]glucose (Ra), percent carbon recycling, mean transit time (the average sojourn time of a glucose molecule in the glucose mass) and the glucose mass (Mmin) were determined according to the graphical procedures of Katz et al. (12). The replacement rate or production of glucose in the postabsorptive animal can be defined biochemically as being equal to the rate of hydrolysis of glucose 6-phosphate by glucose-6-phosphatase (13). The rate of uptake of glucose (utilization) is essentially equal to the rate of glucose phosphorylation (13). In these experiments the assumption was made that the glucose replacement rate (Rg) can be measured in fed fish since the diet of these animals contains minimal carbohydrate. Equations used to determine the above parameters are listed in Table 1. In these procedures, developed for mammals, the area under the linear specific activity curve is determined from time 0 to time t when the specific activity is 1% of that at time 0. Since the glucose replacement rate in fish is low, the time required to approach this specific activity is considerably longer than the feasible duration of a fish experiment. To overcome this problem, the steady-state experimental data points (a minimum of four points falling on a first-order kinetic semilogarithmic plot) are fitted by an exponential least-squares analysis (CompuCorp Statistician 344) and an extrapolation is made to time t. The area under the specific activity curve is then determined as previously described (8). Mean transit time (t) is determined by transforming the single injection specific activity curve to one representing a constant infusion (12). After normalizing units, the area bounded by the constant infusion curve, its plateau, and the ordinate represents the mean transit time.

Rates of glucose production (Rg1) and utilization (Rg2) for nonsteady-state isotope-dilution experiments were calculated using the equations (9) which are presented in Table 1. We are aware that relatively few points are used in these calculations (Figs. 3–5); however, the number of blood samples taken was limited by the small blood volume of these fish. The results are expressed as mg glucose/min per 100 g body wt by multiplying both
$R_1$, and $R_2$ (mg/min per ml plasma) by the estimated glucose space per 100 g (Table 1).

RESULTS

There is no significant difference in plasma glucose concentrations in fed (48±8 mg/100 ml) and fasted (43±8 mg/100 ml) kelp bass (Table 2). In both groups the range of glucose values is wide (6–159 and 4–100 mg/100 ml). This variability suggests that the regulatory mechanisms for controlling blood sugar in kelp bass are not as precise as those found in mammals, although the average plasma levels can be maintained for up to 40 days (the length of this study) without dietary input. There appeared to be no correlation between glucose concentration and the time of year.

Figure 2 illustrates the variations of plasma glucose which occur in four individual fish over a 24-h time period. The fluctuations are pronounced and can change by as much as 93 mg/100 ml during 2 h (Fig. 2, fish 2). Although marked variations occur in the four fish shown, there are periods of comparative stability in the plasma glucose levels; e.g., in fish 3, a variation of only 17 mg/100 ml occurs over a period of 14 h. This implies the existence of steady-state conditions (i.e., glucose production is equal to uptake) during these periods. The number of individuals was insufficient to establish any diurnal patterns.

A series of isotope dilution experiments using [6-$^3$H,6-$^{14}$C]glucose was undertaken in fed and fasted kelp bass to determine simultaneously the glucose replacement rate ($R_n$) with [6-$^3$H]glucose, the apparent glucose replacement rate ($R_a$) with [6-$^{14}$C]glucose, the minimal glucose mass ($M_{min}$) with both isotopes, the glucose transit times (i) for both [6-$^3$H]glucose and [6-$^{14}$C]glucose, the glucose space ($G_s$), and the percent carbon recycling. Table 3 represents the results of experiments in which the blood sugar of the fish remained relatively constant and for which steady-state conditions were assumed. For comparison, the same metabolic parameters are presented for rats having the same weight range as the kelp bass studied.

Although the mean values for $R_a$ are different in fed and fasted kelp bass (0.035±0.006 vs. 0.025±0.003 mg/min per 100 g body wt), these differences are not statistically significant. The overall mean for all experiments is 0.029±0.003 mg/min per 100 g. The $R_n$ is markedly lower than that measured in postabsorptive rats of similar weight (0.630±0.020 mg/min per 100 g) (7). The apparent rate of glucose replacement ($R_a$) is likewise not significantly different in fed and fasted fish (0.025±0.005 vs. 0.026±0.003 mg/min per 100 g) nor does the overall mean $R_a$ (0.030±0.003) differ from the overall mean $R_n$ (0.029±0.003). As would be expected from the similar values for $R_n$ and $R_a$, the mean transit times (i) for both [6-$^3$H]glucose and [6-$^{14}$C]glucose are not significantly different. Glucose carbon recycling, determined from the difference in $R_n$ and $R_a$ (Table 1), is nonexistent in most of these resting fish in contrast to that observed in rats (19–26%) and rabbits (16–21%) (6, 8). Furthermore glucose carbon recycling is not elevated by fasting even after 40 days. The calculated carbon recycling reported in Table 3 (fish 2, 4, 7, and 10) may well result from the error inherent in graphically determining the replacement rate.

The minimal glucose mass per 100 g body wt is not significantly different in fed and fasted fish, suggesting that the glucose mass is maintained during fasting. The glucose space is also similar (19±4 vs. 15±4 ml/100 g), although considerable variation does exist due to the variability in blood glucose levels. Published values for extracellular space in fish (inulin space, 18% (15); sucrose space, 15.4% (23)) agree reasonably well with our measurements.

In most of the isotope-dilution experiments, plasma glucose levels fluctuated during the sampling period indicating an imbalance in glucose production and utilization (i.e., nonsteady state). Three such experiments

**TABLE 2. Plasma glucose levels in fed and fasted kelp bass**

<table>
<thead>
<tr>
<th></th>
<th>mg/100 ml</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed (28)</td>
<td>48±8</td>
<td>6-159</td>
</tr>
<tr>
<td>Fasted (15)</td>
<td>43±8</td>
<td>4-100</td>
</tr>
</tbody>
</table>

Values are means ± standard error of the mean. Number of determinations are in parentheses. In fed fish the last feeding was from 1 to 13 days before the experiment, fasted fish were deprived of food from 20 to 42 days before the experiment.
TABLE 3. Parameters of glucose metabolism in fed and fasted kelp bass and postabsorptive rats determined following a single injection of [6-3H,6-14C]glucose

<table>
<thead>
<tr>
<th></th>
<th>Wt, g</th>
<th>Days without Food</th>
<th>Plasma Glucose, mg/100 ml</th>
<th>Replacement Rate, mg/min per 100 g</th>
<th>Recycling, %</th>
<th>Mwater, mg/100 g</th>
<th>Glucose Space, ml/100 g</th>
<th>Transit Time, min</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R₆₉</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed fish</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>277</td>
<td>9</td>
<td>25 ± 3</td>
<td>0.032</td>
<td>0.035</td>
<td>0</td>
<td>6.4</td>
<td>96</td>
</tr>
<tr>
<td>2</td>
<td>269</td>
<td>9</td>
<td>34 ± 4</td>
<td>0.022</td>
<td>0.021</td>
<td>5</td>
<td>4.8</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>175</td>
<td>5</td>
<td>48 ± 5</td>
<td>0.036</td>
<td>0.038</td>
<td>0</td>
<td>5.0</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>169</td>
<td>2</td>
<td>31 ± 3</td>
<td>0.051</td>
<td>0.045</td>
<td>13</td>
<td>8.0</td>
<td>20</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>0.035</td>
<td>0.035</td>
<td>6.1 ± 0.7</td>
<td>19 ± 4</td>
<td>178 ± 18</td>
</tr>
<tr>
<td>Fasted fish</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>5</td>
<td>355</td>
<td>25</td>
<td>30 ± 2</td>
<td>0.022</td>
<td></td>
<td>5.1</td>
<td>17</td>
<td>234</td>
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<tr>
<td>6</td>
<td>356</td>
<td>40</td>
<td>75 ± 4</td>
<td>0.022</td>
<td>0.025</td>
<td>0</td>
<td>6.8</td>
<td>9</td>
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<tr>
<td>7</td>
<td>261</td>
<td>42</td>
<td>16 ± 2</td>
<td>0.039</td>
<td>0.032</td>
<td>18</td>
<td>5.3</td>
<td>33</td>
</tr>
<tr>
<td>8</td>
<td>235</td>
<td>40</td>
<td>51 ± 1</td>
<td>0.021</td>
<td>0.025</td>
<td>0</td>
<td>5.5</td>
<td>11</td>
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<tr>
<td>9</td>
<td>186</td>
<td>40</td>
<td>55 ± 2</td>
<td>0.019</td>
<td>0.024</td>
<td>0</td>
<td>5.7</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>146</td>
<td>31</td>
<td>37 ± 3</td>
<td>0.024</td>
<td>0.022</td>
<td>10</td>
<td>3.3</td>
<td>9</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>0.025</td>
<td>0.026</td>
<td>5.3 ± 0.5</td>
<td>15 ± 4</td>
<td>250 ± 32</td>
</tr>
<tr>
<td>Overall mean</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.029</td>
<td>0.030</td>
<td>5.6 ± 0.4</td>
<td>17 ± 3</td>
<td>209 ± 21</td>
</tr>
<tr>
<td>Rat</td>
<td>250-275</td>
<td>110 ± 4</td>
<td>0.630</td>
<td>28 ± 2</td>
<td>24.5 ± 1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± standard error of the mean. * Mean value for 4-h experiment. † R₆ and Rₛ determined from [6-3H]glucose and [6-14C]glucose, respectively. ‡ Minimal mass determined from [6-3H]glucose. § Data taken from Dunn et al. (7).

are presented in Figs. 3–5. Calculations of nonsteady-state glucose production and uptake were carried out with samples taken after 60 min to eliminate early specific activity changes due primarily to distribution of the administered isotopic glucose in the glucose space. Tritiated allose, a nonutilizable sugar, is well distributed within 30–60 min postinjection in these fish (unpublished results).

In Fig. 3, a developing hyperglycemia is observed between 60 and 120 min with a plateau attained at 180 min. This plateau persists for the duration of the experiment. The plasma glucose specific activity decreases continuously through the experiment. The calculated rates of glucose production are elevated (0.185 vs. 0.029 ± 0.003 mg/min per 100 g) between 60 and 240 min, with no measurable peripheral uptake of glucose until 180 min. Again the increased hepatic glucose production relative to uptake causes the developing hyperglycemia. The hepatic glucose production drops between 120 and 180 min and then remains relatively stable until 240 min when it falls to approximately one-half the rate of uptake.

In Fig. 5, the plasma glucose level increases between 30 and 120 min and attains a plateau lasting 60 min. A decrease in concentration occurs between 180 and 240 min following by a second plateau. During this period of increasing plasma glucose levels, specific activity falls, reaching a plateau value between 180 and 240 min. This plateau is then followed by a second decrease. The calculated glucose production rate is high between 60 and 120 min. During this same interval, there is no measurable glucose uptake. Thus the elevation in plasma glucose levels is caused by increased production. Between 120 and 180 min, glucose uptake increases from 0 to 0.067 mg/min per 100 g, causing the observed plateau in plasma glucose concentration. Subsequently, hepatic glucose production drops to zero and, along with a further elevation in uptake, results in a fall in plasma glucose levels (180–240 min). Increased production, coupled with a decrease in uptake, then brings about the second plateau in glucose concentration (240–280 min).

## DISCUSSION

The mean plasma glucose concentration in kelp bass (48 mg/100 ml) is significantly lower than in mammals.
and exhibits no apparent seasonally induced variation. The range of glucose levels in fed fish is wide (6-159 mg/100 ml) and is not changed by prolonged fasting (4-100 mg/100 ml). That fish fasted up to 40 days are in fact able to maintain plasma glucose levels similar to those in fed individuals is indicative of a regulatory ability. Most probably, prolonged starvation (greater than 40 days) would be necessary to induce a significant fall in plasma glucose in these fish. Even after 151 days without food, the plasma glucose concentration does not decrease in goldfish (2). In yellow eels, a decline in glucose is noted after 47 days of fasting with a subsequent increase 49 days later (3).

Fluctuations in glucose levels over a 24-h period (Fig. 2) also suggest that the type of rapid, precise hormonal and biochemical control of circulating glucose levels characteristic of mammals is not well developed in fish. In kelp bass, elevated plasma glucose levels (100 mg/100 ml) induced by an intra-arterial glucose load (0.1 g/kg) dropped to preload levels only after a 10-h delay (unpublished results), again suggesting that regulatory responses in these organisms require hours rather than minutes as in mammals (4). Similar results were found by Tashima and Cahill in the teleost \textit{Opsanus tau} (21) and Thorpe and Ince in both pike (22) and silver eels (11).

The isotopic data obtained from kelp bass under nonsteady-state conditions gives some insight into the timing of physiologically induced alterations in hepatic glucose production and uptake by peripheral tissues in response to changing blood sugar concentrations. A 1- to 2-h lag occurs between elevation in blood glucose and

![Figure 3](image1.png)

\textbf{FIG. 3.} Nonsteady-state experiment in intact kelp bass showing plasma glucose concentrations, plasma glucose specific activity (\(^{3}H\)), and calculated rates of hepatic glucose production and peripheral uptake.

![Figure 4](image2.png)

\textbf{FIG. 4.} Nonsteady-state experiment in intact kelp bass showing plasma glucose concentrations, plasma glucose specific activity (\(^{3}H\)), and calculated rates of hepatic glucose production and peripheral uptake.
GLUCOSE TURNOVER IN FED AND FASTED FISH

MINUTES

Fig. 5. Nonsteady-state experiment in intact kelp bass showing plasma glucose concentrations, plasma glucose specific activity (3H), and calculated rates of hepatic glucose production and peripheral uptake.

increased glucose uptake (Figs. 3–5). Similarly, a shutdown in glucose production also takes place 1–2 h after elevation of the plasma glucose concentration (Fig. 5). In mammals, alterations in plasma glucose levels brought about by hormone or glucose administration result in rapid, nearly simultaneous counterregulatory changes in glucose production and uptake (4). In contrast, the described changes in glucose production and peripheral uptake in kelp bass are not only slow but are separated from each other by as much as 1 h. Our findings are in harmony with the observed time of onset of action of insulin and glucagon on plasma glucose concentrations in fish. Ince and Thorpe (11) reported that an initial hypoglycemic response to intravascular codfish insulin in eels (Anguilla anguilla) occurs 30 min after injection, with maximal effects requiring 9 h. A return to normal plasma levels takes place 48 h postinjection. Similar doses of codfish insulin administered to pike (Esox lucius) required 3–6 h to produce a hypoglycemia which persisted for the duration of the experiment (168 h) (22). Bovine glucagon given intra-arterially in pike (23) (1 and 2 mg/kg) elicited a hyperglycemic response within 30 min and lasted up to 6 h. In Anguilla, 3 h were required to produce a glucagon-induced hyperglycemia; however, the hormone was introduced intraperitoneally (11). Fish then do seem capable of responding slowly to hormones as evidenced by changing levels of circulating glucose.

Steady-state conditions can exist in fed and fasted kelp bass as evidenced by periods during which the blood sugar remains relatively stable. These intervals are seen in animals observed for 24 h (Fig. 2) as well as during the shorter nonsteady-state isotope experiments (Figs. 3–5). [6-3H,6-14C]glucose was used to estimate steady-state glucose replacement rates and the extent of carbon recycling occurring according to the methods of Katz et al. (12). In these procedures, the assumption is made that the tritium in position six of glucose is irreversibly lost to body water as a result of hepatic conversion of pyruvate to phosphoenol pyruvate during the carboxylation of pyruvate to oxaloacetate and interconversion of malate and fumarate. Tritium loss can also occur as a result of transamination of labeled pyruvate (5). The use of [14C]glucose results in underestimation of the extent of carbon recycling owing to the recycling of labeled carbon into newly synthesized glucose. The difference in R surf and R surf reflects the extent of carbon recycling.

Glucose turnover in kelp bass is 1/22 and 1/13 of that found in rats and rabbits, respectively, under similar experimental conditions (7, 8). This is not unexpected since the metabolic rate (oxygen consumption) of poikilotherms is considerably less than that of homeotherms (20). Fasting for up to 40 days does not significantly alter R surf, although the mean value is lower. It is possible that in a given fish prolonged fasting may lower the rate of glucose production and uptake. In our experiments, however, the observed variation in the glucose replacement rate among the fed and fasted groups may obscure small changes induced by fasting in any individual.

Our results suggest that carbon recycling is nonexistent or very low in these resting kelp bass even after 40 days of fasting. In rats under similar resting conditions, there is a significant difference in R surf and R surf and indicates that carbon recycling may account for 19–26% of the total glucose production (6). Increased carbon recycling may occur if these fish were exercised. Black et al. (16) observed increasing plasma lactate levels following extensive exercise.

The reincorporation of 14C-labeled carbon, however, may underestimate the contribution of recycled glucose catabolites to glucose production (13). Dilution of recycled 14C with unlabeled carbon may occur at the level of oxaloacetate since this compound is an intermediate of both gluconeogenesis and the tricarboxylic acid cycle. Further dilution can occur if there is a high rate of fatty acid oxidation. We have shown that extensive amino acid gluconeogenesis takes place in kelp bass (unpublished results). It is possible that unlabeled amino acid carbon entering gluconeogenic pathways could also ob-
secure measurement of carbon recycling with [14C]glucose.

We thank Dr. R. Zimmer and the staff of the Catalina Marine Science Center for their cooperation and the use of laboratory facilities.

REFERENCES