Distinct foraging strategies generated by single-action behavioural flexibility

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Animals respond to a changing environment by adjusting their behaviour. This behavioural flexibility often involves choosing between multiple potential actions. However, little is known about how a single action can be used to achieve strategically distinct functions. Here we show that the omnivorous nematode, *Pristionchus pacificus,* optimizes its mixed-resource diet by prioritizing either predatory or territorial outcomes of biting the bacterivorous nematode, *Caenorhabditis elegans.* To determine biting incentive, *P. pacificus* assesses success probabilities and net energetic gains of treating *C. elegans* as prey, and alternatively as a competitor for bacterial food. Considering both predatory and territorial biting outcomes enables *P. pacificus* to pivot to territorial aggression when predation is difficult. In addition to using these outcomes to valuate biting incentive, *P. pacificus* designates the more lucrative outcome as its objective for biting. This biting subgoal then recruits a search subgoal that specifically promotes predatory or territorial biting, thereby unifying behaviour towards a singular foraging strategy. Furthermore, we identified the invertebrate norepinephrine analogue, octopamine, as critical for switching between predatory and territorial strategies. Collectively, our results demonstrate that behavioural flexibility on the single-action level is achieved by a hierarchy of goals and subgoals that valuate and choose from various outcome choices.

Behavioural flexibility reflects the ability of an individual animal to adjust its behaviour in direct response to environmental stimuli. Studies of behavioural flexibility have generally focused on how animals select appropriate actions, with emphasis on goal-directed decision-making[1,2]. Here, actions are chosen based on an internal causative model of how actions lead to outcomes and how outcomes lead to rewards[2,3,4]. This internal model of contingencies allows animals to adapt quickly to new conditions and plan for delayed rewards. To test sensitivity to changes in outcome or reward contingencies, decision tasks often require an animal to choose between two alternative action choices (i.e. action vs. inaction, left vs. right lever)[4,5]. Implicit in this experimental approach is the suggestion that behavioural flexibility depends on the availability of multiple potential actions. However, very little is known of the behavioural flexibility that can be achieved using only a single action.

We surmise that one way to confer behavioural choice without multiple actions is to associate multiple potential outcomes with a single action. Probabilistic outcomes are featured in neuroeconomic theories of decisions under risk[6], such as expected utility theory[7] and prospect theory[8]. In these theories, each potential outcome is associated with a subjective value, which is then weighted in some way by the probability of achieving that outcome as a result of executing an action. Then, the action choice with the highest sum of probability-weighted outcome values is selected. Humans have been shown to valuate actions in this way, particularly when asked to choose between lotteries that have different probabilities and magnitudes of monetary outcomes (gains, losses)[6,9]. However, it remains unclear whether animals use probability-weighted outcome values to also determine which outcome should be pursued for a particular action.

Unlike simple lotteries, naturalistic actions do not always have independent outcomes, and multiple outcomes may occur simultaneously. For example, when a predator kills a prey that

is also a competitor (intraguild predation), it achieves dual benefits of prey food and reduced competition for a shared resource[10]. However, it is often difficult to ascertain whether reduced competition is intentional or a side effect of predation. Previous approaches include comparing the uneaten proportion of killed competitor prey to that of killed non-competitor prey[11]. However, this method does not evaluate non-lethal attacks, which could be failed predatory attempts or deliberate aggression against competitors. In foraging theory, a more inclusive metric of predatory diet choice is the probability of an attack given an encounter with prey[12]. By accounting for prey mobility[13] and simultaneous encounters with multiple food choices[14,15], foraging theory can be adapted to intraguild predation to predict whether the predatory or competitive outcome of attack should be prioritized.

Here, we investigated the ability of the omnivorous nematode, *Pristionchus pacificus* (Fig. 1a), to evaluate multiple food-related outcomes associated with biting the bacterivorous nematode, *Caenorhabditis elegans* (Fig. 1b,c). Both *P. pacificus* and *C. elegans* consume bacteria, but *P. pacificus* can also use its teeth (which *C. elegans* lack) (Extended Data Fig. 1a-c) to fatally bite and eat *C. elegans*[16,17]. Although *P. pacificus* bites *C. elegans* of all stages, these bites typically kill larvae but rarely kill adults[17] (Extended Data Fig. 1d-g, Supplementary Video 1,2). We hypothesized that the non-lethal adult-targeted bites are not failed predatory attempts, but instead intentional aggressive efforts to expel competitors from a bacterial territory. Here we use multivariate behavioural assays informed by neuroeconomics and foraging theory to demonstrate that *P. pacificus* uses hierarchical goal-directed decision-making to flexibly choose between predatory and territorial strategies for biting.

Probabilities and values of biting outcomes

We first assessed probabilities of outcomes associated with a *P. pacificus* bite. Predatory outcomes entail lethal capture and consumption of prey, while territorial outcomes involve removal of competitors from a territory. To characterize capture success, we confined *P. pacificus* and *C. elegans* in a small arena and measured how often individual bites attached onto and killed *C. elegans*. We found that larva-targeted bites often resulted in attachment and death, whereas adult-targeted bites attached less and rarely killed (stage × capture, $p < 0.0001$) (Fig. 1d, Extended Data Fig. 2a,b). Even when the same adult target was repeatedly bitten, killing required ~ 6 hours and ~25 bites (Extended Data Fig. 2c,d). Additionally, most larva-targeted bites led to feeding, whereas the majority of adult-targeted bites that occurred on a bacterial patch prompted *C. elegans* to leave (Fig. 1e,f, Extended Data Fig. 2e-l, Supplementary Video 1,3). Since successful predation also eliminates competition, larva-targeted biting simultaneously achieves both predation and territoriality with relative ease. With adult-targeted biting, predation is more difficult than successful territorial defence.

Since a *C. elegans* adult (1 mm) is much larger than a larva (200 μm) and thus may contain more food mass, the additional effort required to kill adult prey may yield a net energetic gain in the long run. To measure long-term net energy yield of various single-food diets, *P. pacificus* were allowed to feed freely on excess bacteria (*E. coli* OP50), larval *C. elegans*, or adult *C. elegans* for 6 hours before being stained for fat stores with the lipophilic dye Oil Red O (see Methods). *P. pacificus* fed with bacteria displayed the most stained fat, followed by adult-fed and then larva-fed *P. pacificus* (Fig. 1g, Extended Data Fig. 3a-d). Thus, bacteria-based diets are associated with higher energy yields than prey-based diets. Further, given enough time for successful predation, a diet comprised of adult *C. elegans* is more efficient than a larva-based diet.

Although adult prey is more valuable than larval prey in the long term, *P. pacificus* may discount delayed rewards[19] to avoid food deprivation. To assess short-term food preference, we placed *P. pacificus* in one of two plentiful, neighbouring food patches and then checked whether *P. pacificus* switched to the alternative food patch (see Methods). By comparing switching probabilities (Fig. 1h), we ranked *P. pacificus* food choices: bacteria > larval *C. elegans* > adult *C. elegans.* Preference for bacteria over larvae has been previously shown[17]. Contrary to long-term food value, *P. pacificus* preferred larval over adult prey (Fig. 1h). Notably, inverse switches (a→b, b→a) had combined probabilities less than 1 (Fig. 1h), which is consistent with previous reports that nematodes tend to stay within a food patch[18] , and with foraging theory that discounts food choices by its travel time[14]. Overall, preference for easily consumed foods and for closer foods suggest that *P. pacificus* prefers immediate over delayed food rewards.

We next explored the potential competitive benefits associated with the territorial outcome of biting. To compare their ability to exploit bacteria, we placed an adult *C. elegans* and an adult *P. pacificus* onto separate identical patches of GFP-labelled bacteria, and then measured bacterial fluorescence at 12 and 24 hours. We found that adult *C. elegans* consumed bacteria ~1.5x faster than adult *P. pacificus* at both time points (Fig. 1i). Adult *C. elegans* laid eggs on the bacterial patch that began hatching at 12 hours, with a range of 20 to 62 larvae present by 24 hours. However, bacterial consumption rate does not increase between 12 and 24 hours, and we found no correlation between number of larvae and bacteria consumed (Pearson's $r = 0.2480$, $p = 0.8066$) (Fig. 1i, Extended Data Fig. 3e,f). These results show that adult *C. elegans* more efficiently exploits bacteria and may outcompete *P. pacificus* for bacteria, but larvae pose a negligible short-term term.

Using biting outcome probabilities, food values, and competitive values, we predicted how *P. pacificus* should adjust bite choice to maximize food rewards (Fig. 1j, see Methods). To focus on the decision between biting outcomes, rather than on prey preference, we contrived food choices such that *P. pacificus* never encounters larval and adult *C. elegans* in the same arena. While keeping *C. elegans* abundance constant (1 adult or ~100 larvae), we varied bacterial abundance. We subdivided bacterial abundance into three behaviourally defined subranges: 1) in the 'negligible' subrange, *P. pacificus* considers bacteria too meagre too exploit, 2) in the 'scarce' subrange, *P. pacificus* exploits bacteria, but requires biting to increase food supply, and 3) in the 'plentiful' subrange, *P. pacificus* has excess preferred food and does not bite (Fig. 1j). We estimated the bounds of these subranges as well as how biting value should change across bacterial abundance, depending on the expected outcome and *C. elegans* target.

Since the purpose of predation is to kill prey for food, the value of the predatory biting outcome should be highest when bacterial abundance is negligible, and then monotonically decrease as the abundance of its preferred food, bacteria, increases (Fig. 1j). This is consistent with previous reports that *P. pacificus* bites larvae less when bacteria are present than when absent[17]. We used relative fat-staining values (Fig. 1g) and switching probabilities (Fig. 1h) to estimate predatory biting value over the 'negligible' and 'scarce' subrange (Fig. 1j, see Methods). By definition, the 'plentiful' subrange begins where predatory biting value reaches zero. Even though predatory biting value is higher for adult prey than for larval prey over the 'negligible' subrange, *P. pacificus* should drop adult prey from its diet at a lower bacterial abundance than for larval prey due to preference for immediate food rewards (Fig. 1j, see Methods).

In contrast, the purpose of territoriality is to remove competitors from a territory, and therefore the value of the territorial biting outcome depends on an existent bacterial territory and on competitive pressure. Without a bacterial territory to defend, territorial biting incentive is non-existent over the 'negligible' subrange (Fig. 1j). However, territorial biting value should sharply peak at the lower bound of the 'scarce' subrange and then monotonically decrease as scarcity-induced competitive pressure decreases (Fig. 1j). This is similar to other energy cost models of feeding territoriality[20]. We used *C. elegans* bacterial consumption rate to calculate the peak territorial biting value, and we estimated the 'scarce' range to be wider to account for bacterial loss to competitors (Fig. 1j, see Methods). Therefore, the 'plentiful' subrange represents excess bacterial abundance that accommodates both *P. pacificus* and adult *C. elegans.* Notably, predatory and territorial value functions have similar shapes across 'scarce' and 'plentiful' subranges, but differ in whether the value over the 'negligible' subrange is higher or lower than adjacent values in the 'scarce' subrange (Fig. 1j).

By multiplying these predatory and territorial biting value functions by their corresponding outcome probabilities, we predicted that either very low probability or utility would strongly disfavour one outcome for each type of encounter (larva vs. adult) (Fig. 1j). Larvae consume very little bacteria making them a negligible short-term competitive threat. Because of this low territorial utility, this model predicts that *P. pacificus* should select the predatory outcome as its goal for biting larval *C. elegans*. For biting adult *C. elegans, P. pacificus* should strategically choose the territorial outcome based on the low probability of killing adult prey.

Territorial consequences of biting

Our biting choice predictions are predicated on the validity of both predatory and territorial biting outcomes, but only the former has been demonstrated in *P. pacificus*[16,17]. Before we could assess biting choice, we first needed to confirm the long-term consequences of the territorial biting outcome. To examine bite-induced patch-leaving, we placed adult *C. elegans* with or without *P. pacificus* on a small bacterial patch, and recorded *C. elegans* residence time inside the patch. Without *P. pacificus*, *C. elegans* mostly resided fully within the patch, but introduction of *P. pacificus* immediately caused *C. elegans* to reduce patch residence time (Fig. 2a,b). After 6 hours of biting exposure, *C. elegans* almost completely avoided entering the patch, often with only its nose at the outer fringes (Fig. 2a,c). Furthermore, avoidance time per bite increased fivefold (Extended Data Fig. 3h), suggesting that *C. elegans* was conditioned to associate the patch with danger. By interfering with *C. elegans* access to bacteria, *P. pacificus* can defend against depletion of its bacterial territory. Furthermore, biting for territorial defence becomes more efficient over time.

We speculated that this biting-induced patch avoidance would force *C. elegans* to lay eggs away from bacteria. To test this, we developed an egg distribution assay (see Methods, Extended Data Fig. 4a-d) to measure where eggs were laid relative to a scarce bacterial patch over a 7-hour period (before eggs hatched). When *C. elegans* or *P. pacificus* adults laid eggs separately from each other, most eggs were deposited inside the patch, indicating absence of within-species territoriality (Fig. 2d). In mixed groups, however, *C. elegans* laid more eggs off the patch, shifting the distribution of eggs away from the patch (Fig. 2d,e, Extended Data Fig. 4e,f). Meanwhile, the distribution of *P. pacificus* eggs was unaffected (species × mix, $p < 0.0001$) (Fig. 2d). The total number of *C. elegans* eggs also remained unchanged, indicating that

P. pacificus rarely eats *C. elegans* eggs (Extended Data Fig. 4g,h). Therefore, biting interferes with *C. elegans* preference for laying eggs within bacteria.

We next asked whether newly hatched *C. elegans* larvae would struggle to find a distant scarce bacterial patch. Since failure to find food within 36 hours induces arrested reproductive development in larvae ('dauer' state) [21], we placed larvae at various distances from a scarce bacterial patch and counted how many found the patch within 36 hours. At 10 mm away, half the larvae failed to find the patch, with increased failure at greater distances (Fig. 2f). To verify that biting causes adult *C. elegans* to lay eggs at these unfavourable distances, we spatially and temporally extended the egg distribution assay to 100 mm and 36 hours, respectively. Introduction of *P. pacificus* reduced the number of *C. elegans* larvae within 10 mm of the bacterial patch by more than half (Fig. 2g). Although *C. elegans* is typically more prolific (Extended Data Fig. 4i), *P. pacificus* progeny outnumbered *C. elegans* progeny within 10 mm of the patch (Fig. 2g). Altogether, these results illustrate how nonlethal biting accrues long-term territorial benefits and increases *P. pacificus* fitness relative to *C. elegans*.

Subgoal-directed biting and search

To unravel whether the territorial benefits of biting adult *C. elegans* are intentional or a serendipitous side effect of failed predation, we experimentally tested our bite choice predictions (Fig. 1j). We evaluated active biting choice by using the probability of a bite given an encounter with *C. elegans*, $p(bite|encounter)$, as a quantitative measure of biting incentive. We then monitored how $p(bite|encounter)$ changed with bacterial abundance parameters (density, diameter) to infer which biting outcome contributed more to biting decisions. As predicted, we observed that larva-targeted bite incentive monotonically decreased as bacterial abundance increased (Spearman's $\rho = -0.58$, $p < 0.001$) (Fig. 3a), matching the predatory biting value

function (Fig. 1j). By contrast, adult-targeted biting incentive was low when bacteria were negligible and high when bacteria were scarce (Fig. 3b), which conforms with the non-monotonic structure (Spearman's $\rho = 0.08$, $p = 0.374$; Hoeffding's $D = 0.03$, $p < 0.001$) of the territorial biting value function (Fig. 1j). Importantly, adult-targeted biting incentive diminishes at a higher bacterial abundance than larva-targeted biting incentive, consistent with our prediction of a wider 'scarce' subrange for the adult-targeted territorial biting value function. To examine the critical bacterial abundance threshold between 'negligible' and 'scarce' subranges (Fig. 1j), we used a low-density bacterial patch that induced patch-staying and patch-leaving behaviours with roughly equal probabilities (Fig. 3c, Extended Data Fig. 5a-c, Supplementary Video 3,4). Using choice variability approach for probing decision-making[22], we segregated encounters into off- and on-patch events to reflect *P. pacificus* decision to ignore or exploit the patch, respectively. Off-patch biting incentive was higher for larva-targeted bites, while on-patch biting incentive was higher for adult-targeted bites (Fig. 3a,b). These results support the hypothesis that *P. pacificus* considers both predatory and territorial biting outcomes in a context-specific manner, and incorporates both *C. elegans* and bacterial information to execute predation against larval *C. elegans* and territorial aggression against adults.

We next explored how *P. pacificus* senses bacteria for adjusting territorial biting incentive. To identify relevant chemosensory neurons, we ablated bilateral pairs of amphid neurons that have exposed cilia at the *P. pacificus* nose (Extended Data Fig. 5d, see Methods). Relative to mock controls, ablation of ASH and AWC neurons decreased biting incentive against adult *C. elegans* on scarce bacterial patches, while ablation of ADL neurons increased biting incentive (Fig. 3d). Although these neurons are poorly understood in *P. pacificus*, studies of homologous neurons in *C. elegans* may offer some clues. The olfactory neuron AWC triggers

local search behaviour upon removal from a bacterial patch[23], and also senses bacteria-related odorants[24,25]. In *C. elegans*, ASH and ADL are involved in avoiding high ambient oxygen[26,27] and migrating to the thick boundary of a bacterial patch, where local oxygen concentration is lower due to higher bacterial metabolism[23]. Recent studies report that *P. pacificus* can similarly distinguish between oxygen levels[29] (albeit via different molecular mechanisms[30]), and mutants with cilia-defective amphid neurons exhibit impaired oxygen responses[31].

In addition to chemosensation, we also probed how mechanosensation of bacteria modulates territorial biting. We measured adult-targeted biting incentive on patches composed of Sephadex gel beads, which mimic the mechanosensory contact of bacteria[33]. Importantly, these beads are inedible and lack the chemical signatures of live bacteria. As with low-density bacterial patches, *P. pacificus* spent less time on a bead patch than on medium- or high-density bacterial patches (Fig. 3c, Extended Data Fig. 5a,e). On-patch biting incentive was high (Fig. 3e), similar to that associated with a low-density bacterial patch of the same size (Fig. 3b). This high biting incentive was not suppressed in larger bead patches (Fig. 3e). *P. pacificus* decreased its residence on a bead patch over time, opposite of what it does on a low-density bacterial patch (Fig. 3f, Extended Data Fig. 5f), suggesting that eating is required to sustain patch exploitation. These results suggest that *P. pacificus* senses some minimal 'bacterial' abundance in these bead patches, and that mechanosensation may be used to detect small amounts of bacteria.

Our results thus far demonstrate that *P. pacificus* flexibly decides between biting and inaction in a context-specific manner, based on expectations of predatory and territorial outcomes. However, it remains to be shown whether *P. pacificus* chooses an outcome to assign as its biting subgoal, or whether having that choice imparts any benefit. While goals are the expected final reward of behaviour and have inherent hedonic value[32], subgoals specify

intermediate objectives of behaviour[41]. To achieve the goal of optimal diet, assigning a biting subgoal may be beneficial if it can coordinate other behaviours to achieve distinct predatory or territorial foraging strategies. We reasoned that predatory and territorial biting subgoals should differ in how they guide *P. pacificus* to search for *C. elegans*, by specifying opposite subordinate search subgoals for managing *C. elegans* movement relative to a bacterial patch. Specifically, the predatory search subgoal should promote capture and immobilisation of *C. elegans* prey before they leave the patch, while the territorial search subgoal should aid expulsion of *C. elegans* from the patch.

We contemplated how these search subgoals should influence search speed on a bacterial patch. Under the predatory search subgoal, *P. pacificus* should minimize search costs since prey are inferior to bacteria, and there is no urgency to hunt while prey reside on the patch. Rather than increase search speed, *P. pacificus* should graze normally on bacteria and opportunistically bite prey during chance encounters. Under the territorial search subgoal, *P. pacificus* should swiftly find and expel intruders to halt rapid loss of preferred food to adult *C. elegans*. To test these predictions, we tracked *P. pacificus*'s location on a scarce bacterial patch that contained or lacked *C. elegans*. Since both nematode species are attracted to the thick boundary of a bacterial patch[23,27,29], we assessed *P. pacificus's* intent to search for *C. elegans* by measuring forward angular movement along the patch circumference (patrol speed) (Supplementary Video 5, see Methods) relative to x-y movement (translational speed). We found that *P. pacificus* exhibited faster patrol and translational speed with adult patch-mates than with larvae or without *C. elegans* (Fig. 3g-i, Extended Data Fig. 5g). Additionally, the ratio of patrol speed to translational speed was higher, signifying that *P. pacificus* directed a greater proportion of its locomotion to patrolling the patch boundary when adult *C. elegans* was present (Fig. 3j). Speed ratio did not

differ between patches with larvae and without *C. elegans* (Fig. 3j), suggesting that grazing on bacteria motivated both exploration patterns. Moreover, speed and speed ratio decreased when *P. pacificus* shared a patch with *C. elegans* that has been conditioned to mostly avoid the patch, (Extended Data Fig. 5h-j). While both larva-targeted and adult-targeted biting incentives were typically high for the tested bacterial patch, patrolling only increased with adult patch-mates and therefore did not support biting in general. Thus, the territorial biting subgoal recruits patrolling behaviour to specifically support its search subgoal of expelling *C. elegans* from a bacterial territory.

Since *P. pacificus* must stop feeding on prey to resume search for other prey, we reasoned that one way to increase prey encounters without increasing search cost is to reduce feeding time. Prey are mobile, so while *P. pacificus* feeds on one prey, other prey could be dispersing, especially when bacterial abundance is not high enough to retain prey on the patch. To limit dispersal, *P. pacificus* can kill prey to immobilize it now and then finish eating it later. To test whether this search tactic supports the predatory search subgoal, we measured the time *P. pacificus* spends feeding on larval prey (~100 in each arena) across various types of bacterial patches. Importantly, larvae were able to escape the arena, making dispersal a real threat of food loss. As expected, we found that prey-feeding time per bite was lower when bacteria were absent or low-density, and higher when bacteria were medium- or high-density (Fig. 3k). If prey-feeding time were closely associated with biting in general rather than with the predatory biting subgoal, then we would expect prey-feeding times that were graded with biting incentive. However, we did not see any difference between prey-feedings times on and off a low-density patch ($p = 0.90558$), or between a medium- and high-density 1 mm diameter patch ($p = 0.99965$) (Fig. 3k), where we have observed significant differences in biting incentive (Fig. 3a). Instead,

feeding times matched *P. pacificus*'s own patch-leaving behaviour (Fig. 2c), which may serve as heuristic for judging other competing nematodes' patch-leaving proclivity. Therefore, *P. pacificus* modulates prey-feeding time to achieve the search subgoal particular to the predatory biting subgoal.

Switching between biting subgoals

We next sought to induce a switch from a predatory biting subgoal to a territorial biting subgoal. Food deprivation is commonly used to increase food reward value in decision-making[34]. We expected that food deprivation would have a multifaceted effect, since multiple food types are involved in *P. pacificus* biting choice. To test this, *P. pacificus* was food-deprived for two hours and then placed with adult *C. elegans* in either a bacteria-free arena or a scarce bacterial patch. Compared to well-fed *P. pacificus*, food-deprived biting incentive increased when bacteria were absent, and decreased on scarce bacteria (hours food-deprived × arena, $p < 0.0001$) (Fig. 4a). This suggests that food deprivation increased valuation of immediate feeding by increasing the value of adult prey in the absence of any other food, and decreased the value of territorial rewards relative to immediate bacterial rewards. However, biting incentive remained higher on scarce bacteria than when bacteria were absent (Fig. 4a), implying that the territorial biting outcome still dominated food-deprived adult-targeted bite choice.

We next used pharmacological methods to identify signalling pathways that regulate territorial biting. To expediently screen a library of 680 neurotransmission-related drugs, we used our egg distribution assay (instead of time-intensive behavioural recordings) to identify candidates that increased territorial biting outcomes. We reasoned that drugs that promoted territorial biting could be indirectly detected as a shift in *C. elegans* egg distribution away from patches (Fig. 2). We did not pursue drugs that reduced territorial outcomes (more eggs on the

patch), since reduced biting could be due to a number of mechanisms unrelated to biting motivation. Using increasingly stringent assays for narrowing the pool of drug candidates (see Methods), we found that treating *P. pacificus* with amisulpride, a dopamine D2 receptor antagonist, either: 1) increased the mean distance from bacteria that *C. elegans* laid their eggs (Fig. 4b), or 2) increased the percent of eggs laid off the bacterial patch (Extended Data Fig. 6a-c). Therefore, we pursued amisulpride as a promising drug for promoting territorial biting.

To ascertain whether amisulpride increases biting in general or in context-specific manner, we next measured adult-targeted biting incentive in amisulpride-treated *P. pacificus* across a range of bacterial conditions. Amisulpride treatment enhanced biting incentive when bacteria were scarce, but had no effect when bacteria were absent or plentiful (Figure 4c). This suggests that amisulpride does not affect general arousal to bite, but is contingent on co-occurrence of bacterial stimuli that signal competitive pressure. Since this result is consistent with an increase in territorial biting incentive, we wondered if a D2 receptor agonist, the opposite action of amisulpride, could result in predatory biting. However, treating *P. pacificus* with the D2 receptor agonist, sumanirole, did not affect biting incentive in any bacterial condition (Extended Data Fig. 6d). Therefore, D2 receptor drugs do not activate decision signals for choosing between predatory or territorial biting subgoals, but may modulate the effort exerted to accomplish the territorial biting subgoal[35].

We next explored drugs that potentially act on the same pathway as D2 receptors. Since knowledge of *P. pacificus* pathways is limited, we consulted known pathways in its well-researched relative, *C. elegans.* In *C. elegans*, absence of bacteria attenuates D2-like receptor signalling (the biological action of amisulpride), which in turn triggers release of octopamine[36], the invertebrate norepinephrine analogue. We exogenously treated *P. pacificus* with octopamine

and found that, like amisulpride, adult-targeted biting incentive increased only when bacteria were scarce (Extended Data Fig. 6e). To determine whether blocking octopamine would conversely result in predatory biting, we treated *P. pacificus* with epinastine, a high-affinity octopamine receptor antagonist. Biting incentive in epinastine-treated *P. pacificus* increased when bacteria were absent and decreased when bacteria were scarce or plentiful (Fig. 4d). As a result, biting incentive monotonically decreased with bacterial abundance (Fig. 4d), matching predatory biting value functions (Fig. 1j). Moreover, epinastine treatment suppressed patrolling (Fig. 4e,f), suggesting that epinastine also affects the search subgoal. Collectively, we found that blocking octopamine was sufficient to inhibit the territorial biting subgoal and its subordinate behaviours, and instead promote a predatory biting subgoal and predatory search speed.

Discussion

We present a model of *P. pacificus* bite-based foraging strategies that is governed by a hierarchy of goals and subgoals. At the top of the goal hierarchy, biting choice is guided by an internal feeding drive, which promotes the overall goal of optimizing mixed-diet food rewards. Below this top-level goal, *P. pacificus* assesses the probabilities and values of predatory and territorial biting outcomes to determine which to prioritize as its biting subgoal. The predatory biting subgoal treats *C. elegans* as a less-preferred food, while the territorial biting subgoal treats *C. elegans* as a competitor for its preferred food, bacteria. After designating the biting subgoal, *P. pacificus* can then recruit a subordinate search subgoal that supports the predatory biting subgoal by limiting prey dispersal, or supports the territorial biting subgoal by patrolling for intruders. By using the biting subgoal to coordinate other behavioural changes, *P. pacificus* ensures that efforts are unified into a cohesive predatory or territorial foraging strategy.

By suggesting that *P. pacificus* selects from multiple potential subgoals for biting, we argue that *P. pacificus* exhibits behavioural flexibility on the single-action level, in addition to the behavioural flexibility associated with having multiple potential actions (or inaction) to choose from. A simpler alternative is that *P. pacificus* considers both predatory and territorial outcomes to valuate biting, but does not explicitly designate one outcome as the biting subgoal. However, we show that search behaviours are consistent with prioritization of a singular biting outcome rather than with biting in general (Fig. 3g-k, Extended Data Fig. 5g-j). Furthermore, both biting and search behaviours switched from territorial to predatory expressions upon treatment with epinastine (Fig. 4d-f). Another alternative motivational structure is that *P. pacificus* decides on a predatory or territorial strategy independently of biting considerations. However, biting is essential for implementing both these strategies, and we show that *P. pacificus* considers the effectiveness of biting in weighting predatory and territoriality outcomes. We provide compelling evidence that *P. pacificus* deliberates between multiple potential purposes for biting, and suggest future investigation into the underlying neural representations.

In addition to behavioural flexibility of biting, we also reveal flexibility in how *P. pacificus* manipulates different phases of foraging to increase frequency of encounter with *C. elegans.* We show that *P. pacificus* appropriately chooses between passive and active modes of search, depending on whether the food object of biting is inferior (prey) or superior (bacteria) in its net energetic gains. Since both modes of search are associated with delayed food rewards, *P. pacificus* search behaviour may contribute to other efforts to use laboratory foraging tasks to determine whether serotonin promotes passive behaviour, or whether it enhances patience or persistence in pursuing delayed rewards[37,38]. Our results also indicate that *P. pacificus* combines stopping of prey-feeding and search locomotion to form a temporally abstract 'search' action, but

still maintains selective control over how different search-related subtasks influence the temporal trajectory of search. While previous studies have previously reported hierarchical temporal structure of nematode behaviour[39,40], here we show that nematodes also exhibit instrumental behavioural hierarchy in which behaviour can be divided into increasingly specific outcome-oriented subgoals and subtasks[41].

Our study demonstrates that a nematode with approximately 300 neurons[42] can solve complex foraging problems in which the pertinent elements have multiple potential roles: *C. elegans* as prey and competitor, bacteria as a food patch for *P. pacificus* and habitat for *C. elegans*, and bite action with predatory and territorial outcomes. Our study of interactions between *P. pacificus*, *C. elegans*, and bacteria offers insight into how to disentangle these dualities to understand intraguild predation on the single predator motivation level[43]. In particular, consideration of *C. elegans* mobility was key for predicting differences in predatory and territorial responses, which echoes previous reports that foraging theory often failed to predict behaviour when prey mobility was not sufficiently accounted for[13]. For example, while *C. elegans* escape from a bite is considered a failure by predatory standards, it can be leveraged for territorial benefit if escape is directed away from a bacterial patch. This territorial benefit is amplified when *C. elegans* becomes conditioned avoid the bacterial patch in a way that resembles how prey seek refuges to avoid predators[44]. While previous studies looked at how intraguild predation induces prey refuge behaviour[45,46], our work presents a complementary perspective of how predators respond to this refuge behaviour. Our study also highlights the importance of prey mobility for interpreting prey-feeding in intraguild predation. Feeding on prey is typically associated with predatory motivation, and previous studies of intraguild predation measured uneaten killed prey to indicate potentially competitive motivation[11]. Similar

suggestions of competitive intent were recently made about *P. pacificus* surplus-killing of *C. elegans* larvae in the absence of bacteria[17,47]. However, we found that the contexts with the highest predatory incentive were associated with reduced prey-feeding time, which runs contrary to classic foraging models that predict lower prey utilization when prey densities are high[48]. This observation is reasonable once we consider that 1) searching for other prey is sequentially dependent on termination of current prey-feeding, 2) prey disperse when bacterial abundance is low, and 3) killed prey are immobile and can be cached for later consumption. Collectively, our use of neuroeconomics, foraging theory, and fine-grained manipulations of foraging contexts illustrate that the multiple motivational states can produce the same behaviour, and that a careful accounting of context is required to appropriately associate motivational states with behavioural expression. Furthermore, our study supports a resurgent effort to reaffirm behavioural interrogation as being equally or more useful than neuroscientific methods for understanding cognitive processes[49], with emphasis on understanding how an animal's responses are relevant to its natural life[50].

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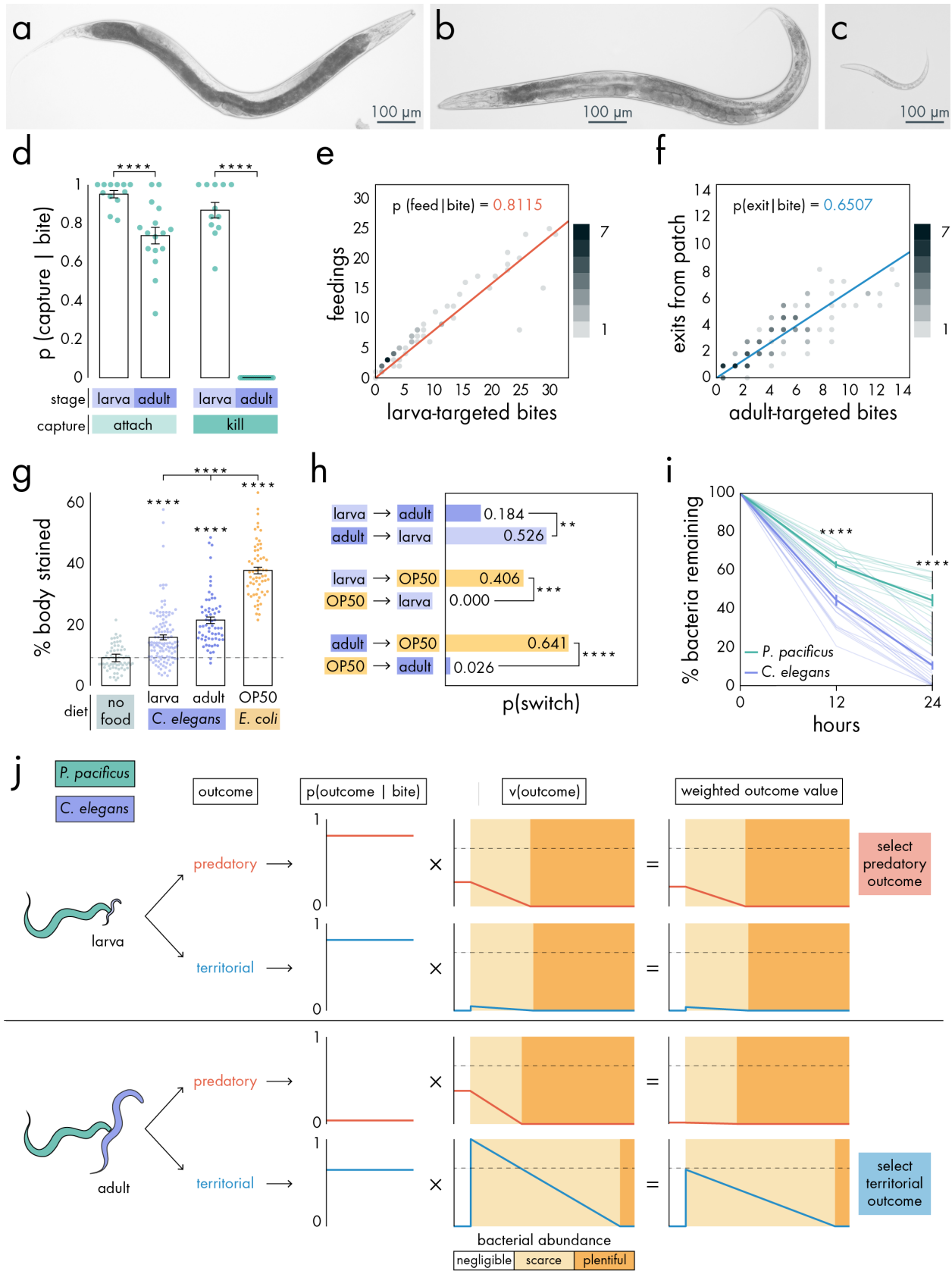
a
100 µm
b
100 µm
c
100 µm
d
p (capture | bite)
****
****
stage
larva
adult
larva
adult
capture
attach
kill
e
p (feed | bite) = 0.8115
feedings
larva-targeted bites
f
p(exit | bite) = 0.6507
exits from patch
adult-targeted bites
g
% body stained
****
****
****
****
diet
no food
larva
adult
OP50
C. elegans
E. coli
h
larva → adult
adult → larva
larva → OP50
OP50 → larva
adult → OP50
OP50 → adult
0.184
0.526
0.406
0.000
0.641
0.026
**
***
****
p(switch)
i
% bacteria remaining
****
****
P. pacificus
C. elegans
hours
j
P. pacificus
C. elegans
outcome
p(outcome | bite)
v(outcome)
weighted outcome value
larva
predatory
territorial
select predatory outcome
adult
predatory
territorial
select territorial outcome
bacterial abundance
negligible
scarce
plentiful

Fig. 1 | Probabilities and values of biting outcomes. a, Young adult *P. pacificus*. **b,** Young dult *C. elegans*. **c,** Larval *C. elegans* (L1 stage). **d,** Capture success (logistic regression, likelihood ratio test, $n_{P.pacificus}$ = 12-16). **e,** Probability of predatory outcome given a larva-targeted bite. **f,** Probability of territorial outcome given an adult-targeted bite. **g,** Oil Red O staining of *P. pacificus* (Games-Howell test, $n_{P.pacificus}$ = 60-117). **h,** Food switching (Fisher's exact test with Benjamini-Hochberg correction, $n_{P.pacificus}$ = 29-39). **i,** Bacterial consumption (Welch's t-test, n_{adult} = 16-23). **j,** Bite choice model (see Methods). Dashed line represents the relative value of *P. pacificus* feeding by itself on plentiful bacteria. All error bars are mean ± 1 s.e.m.

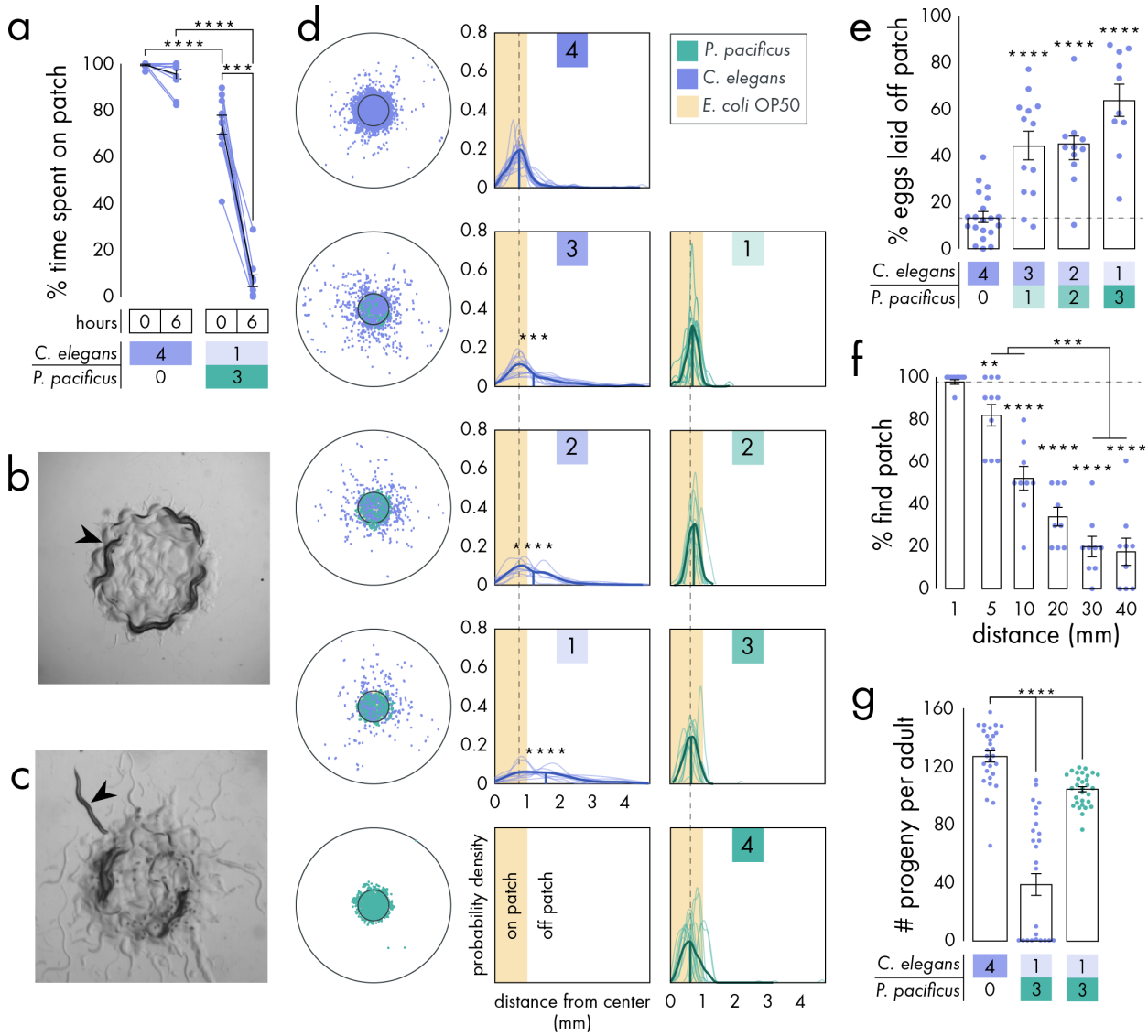
a
% time spent on patch
hours
C. elegans
P. pacificus
b
c
d
P. pacificus
C. elegans
E. coli OP50
probability density
on patch
off patch
distance from center (mm)
e
% eggs laid off patch
f
% find patch
distance (mm)
g
progeny per adult

Fig. 2 | Territorial consequences of biting. a, Adult *C. elegans* patch residence time (Wilcoxon's signed rank test (paired), Welch's t-test (unpaired), $n_{C.elegans} = 11$). **b-c,** Single adult *C. elegans* (black arrow) with three adult *P. pacificus* after **b,** 0 hours, and **c,** 6 hours. **d,** Spatial distributions of eggs laid by *C. elegans* and *P. pacificus* in mixed groups. Inner circle represents the bacterial patch boundary (linear mixed-effects model, $n_{trials} = 10\text{-}20$). **e,** Percentage of eggs laid off patch (logistic regression, $n_{trials} = 10\text{-}20$). **f,** Ability of larval *C. elegans* to find a bacterial patch (logistic regression, $n_{trials} = 9$). **g,** Number of progeny per adult within 10 mm of a small bacterial patch after 36 hours (negative binomial regression, $n_{trials} = 29\text{-}30$)**.** All error bars are mean ± 1 s.e.m.

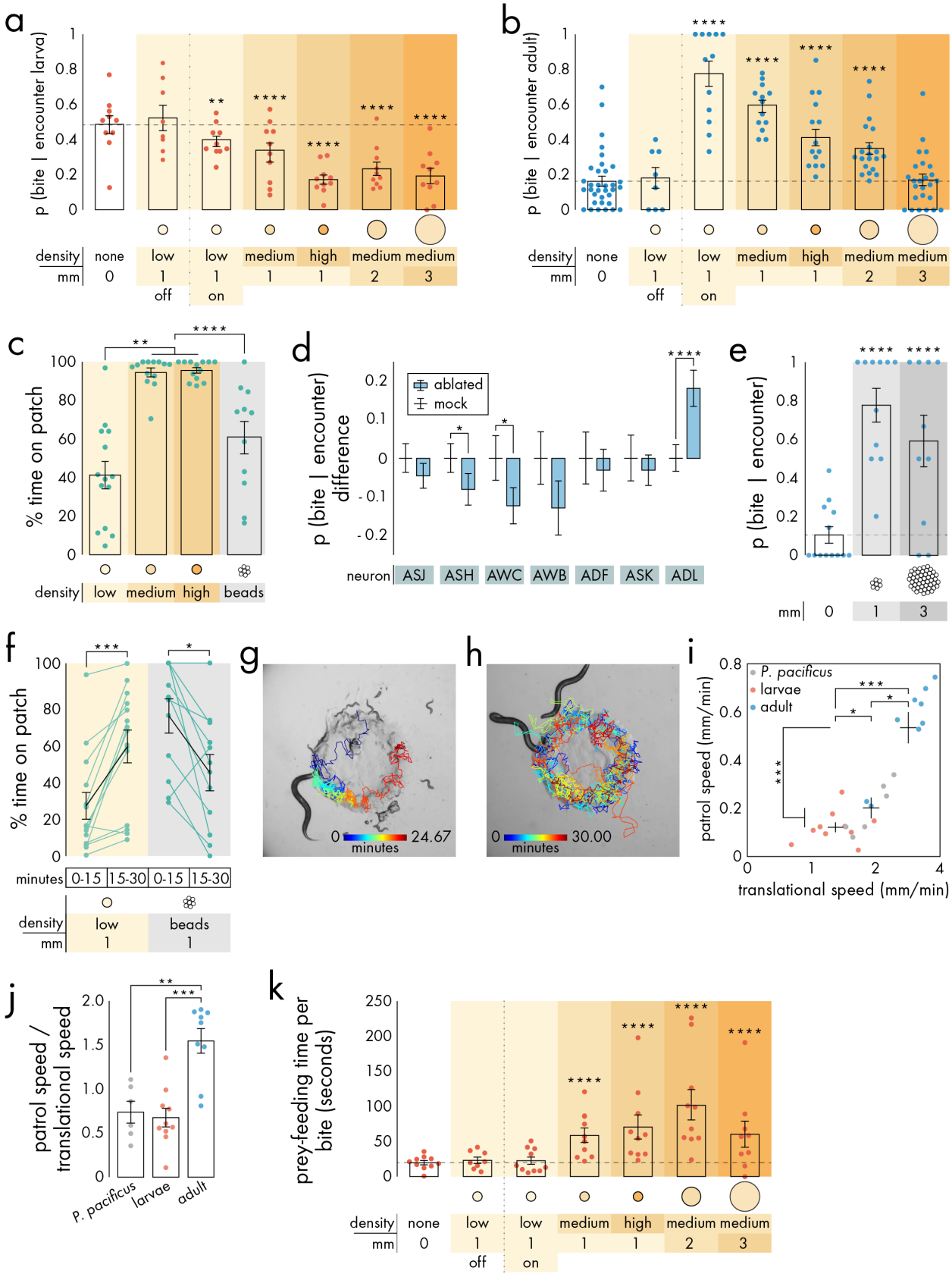
a
p (bite | encounter larva)
density none low low medium high medium medium
mm 0 1 1 1 1 2 3
off on
b
p (bite | encounter adult)
density none low low medium high medium medium
mm 0 1 1 1 1 2 3
off on
c
% time on patch
density low medium high beads
d
p (bite | encounter) difference
ablated
mock
neuron ASJ ASH AWC AWB ADF ASK ADL
e
p (bite | encounter)
mm 0 1 3
f
% time on patch
minutes 0-15 15-30 0-15 15-30
density low beads
mm 1 1
g
0 24.67
minutes
h
0 30.00
minutes
i
P. pacificus
larvae
adult
patrol speed (mm/min)
translational speed (mm/min)
j
patrol speed / translational speed
P. pacificus
larvae
adult
k
prey-feeding time per bite (seconds)
density none low low medium high medium medium
mm 0 1 1 1 1 2 3
off on

Fig. 3 | Subgoal-directed biting and search. a, *p(bite|encounter)* with larval *C. elegans* (logistic regression, $n_{P.pacificus}$ = 9-10). **b,** *p(bite|encounter)* with adult *C. elegans* (logistic regression, $n_{P.pacificus}$ = 12-34)**. c,** *P. pacificus* patch residence time (Dunn's test on log-transformed data, $n_{P.pacificus}$ = 11-14). **d,** Amphid neuron ablations (logistic regression, $n_{P.pacificus}$ = 5-31). **e,** *p(bite|encounter)* on bead patches (logistic regression, $n_{P.pacificus}$ = 7-13). **f,** Patch residence over time (Wilcoxon's signed rank test, $n_{Ppacificus}$ = 11-14). **g-h,** *P. pacificus* tracks on a bacterial patch with **g,** larval *C. elegans* and **h,** adult *C. elegans.* **i,** Patrol speed and translational speed (Tukey's test, $n_{P.pacificus}$ = 6-10). **j,** Speed ratio (Tukey's test, $n_{P.pacificus}$ = 6-10). **k,** Prey-feeding time per bite (linear mixed-effects model, $n_{P.pacificus}$ = 24). All error bars are mean ± 1 s.e.m.

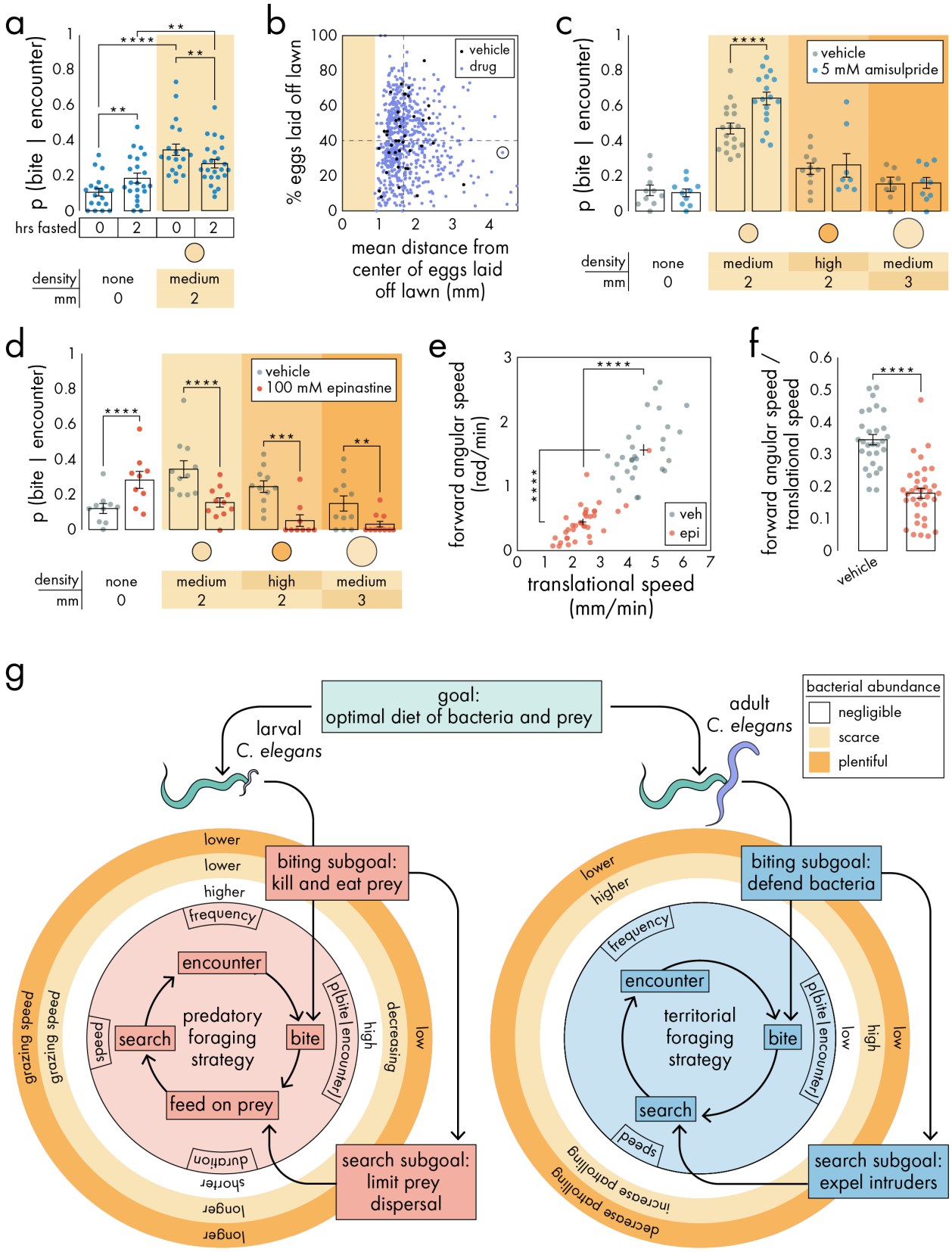
a
p (bite | encounter)
hrs fasted
density
mm
none
medium
b
% eggs laid off lawn
mean distance from center of eggs laid off lawn (mm)
vehicle
drug
c
vehicle
5 mM amisulpride
high
d
vehicle
100 mM epinastine
e
forward angular speed (rad/min)
translational speed (mm/min)
veh
epi
f
forward angular speed / translational speed
vehicle
g
goal: optimal diet of bacteria and prey
larval C. elegans
adult C. elegans
bacterial abundance
negligible
scarce
plentiful
biting subgoal: kill and eat prey
biting subgoal: defend bacteria
search subgoal: limit prey dispersal
search subgoal: expel intruders
predatory foraging strategy
territorial foraging strategy
encounter
search
bite
feed on prey
frequency
duration
speed
p(bite|encounter)
lower
higher
shorter
longer
grazing speed
decreasing
low
high
increase patrolling
decrease patrolling

Fig. 4 | Switching between biting subgoals. a, *p(bite|encounter)* of food-deprived *P. pacficus* (logistic regression, n_{trials} = 19-61). **b,** First pass of drug screen. Dashed lines represent means of control values. **c,** *p(bite|encounter)* of amisulpride-treated *P. pacificus* (logistic regression, n_{trials} = 8-18). **d,** *p(bite|encounter)* of epinastine-treated *P. pacificus* (logistic regression, n_{trials} = 9-11). **e,** Effect of epinastine on patrol speed and translational speed (Wilcoxon's ranked sum test, $n_{Ppacificus}$ = 29-35). **f,** Effect of epinastine on speed ratio (Wilcoxon's ranked sum test, $n_{Ppacificus}$ = 29-35). **g,** Model of *P. pacificus* bite-based foraging strategies. All error bars are mean ± 1 s.e.m.

Methods

Animals

P. pacificus and *C. elegans* were grown on *E. coli* OP50 bacteria and maintained under standard conditions at 20°C [51,52]. Unless otherwise indicated, the *P. pacificus* wild isolate RS5194[53,54] and the standard *C. elegans* N2 strain[52] were used. Other *P. pacificus* wild isolates used includes PS312[51] and RS5275[53,54]. For simplicity, we use 'adult' to refer to the young adult (day 1) stage of both nematode species, and 'larval' to refer to the L1 stage of *C. elegans.* All *P. pacificus* animals used in behaviour were confirmed to have the dual-toothed eurystomatous mouth form (Extended Data Fig. 1a,b), which more efficiently kills larval *C. elegans* compared to single-toothed stenostomatous individuals[17].

Behavioural recordings

Behavioural video recordings were acquired using an optiMOS sCMOS camera (QImaging) and Streampix software. Copper corral arenas were used to keep animals within the field-of-view.

Bacterial patches for behavioural assays

E. coli OP50 were inoculated in LB broth and adjusted to OD_{600} = 0.4, then stored at 4°C for at least two weeks before use (stock). To produce working solutions, the stock culture was either diluted with LB broth, or concentrated by centrifugation (1 ml at 845 rcf for 5 min) and the removal of supernatant. 'Low', 'medium', and 'high' density patches used liquid culture concentrations of OD_{600} = {0.01, 0.30, and 1.00}, respectively (Extended Data Fig. 2f-h). Various volumes of liquid culture were pipetted onto 3% agar NGM plates[55] to produce desired patch diameters (Extended Data Fig. 2f,i,j), and then grown for 20 hours at 20°C. The total number of bacteria pipetted for a high-density, 1 mm patch was less than for a medium-density, 2

mm patch. Fully grown patches were stored at 4°C and then allowed to come to room temperature for 1 hour before use.

Capture success and biting outcomes

Three wild isolates of *P. pacificus* (PS312, RS5275, and RS5194) were assessed for short-term and long-term capture success. Only RS5194 was assessed for biting outcomes.

Short-term capture success was assayed using a modified version of the biting assay described by Wilecki et al.[17]. A single adult *P. pacificus* was placed in a copper-corralled arena (3.2 mm in diameter) with either 8 adult *C. elegans* or ~100 larval *C. elegans*. Biting behaviour was recorded for 30 minutes and subsequently scored for bites, attachments, and kills. Non-attaching bites were identified by the coincidence of: 1) concurrent *P. pacificus* head shortening and stiffening associated with biting (compare Fig. 1a and Extended Data Fig. 1d), and 2) *C. elegans* escape response typical of receiving a hard touch[56]. Attachments, including transient attachments, were identified by disrupted normal locomotion in either nematode caused by the *P. pacificus* mouth being fastened to the *C. elegans* body. This manifested as *C. elegans* thrashing in place while anchored by a *P. pacificus* bite, or dragging of the *P. pacificus* mouth as an adult *C. elegans* attempts to escape from the bite (Supplementary Video 1,2,6). Kills were indicated by a breached cuticle and visible leaking of pseudocoelomic fluid (Extended Data Fig. 1e), ultimately leading to an unresponsive corpse.

Long-term capture success was assayed using the same behavioural setup, but instead a single adult *P. pacificus* was placed with a single adult *C. elegans* for 24 hours. The presence of a killed adult *C. elegans* was checked at 1, 4, 8, and 24 hours.

Biting outcomes were observed using a similar behavioural setup as for short-term capture success (30 minutes, 1 adult *P. pacificus* with ~100 larval *C. elegans* or 1 adult *C. elegans*), but we also tested multiple types of bacterial patches.

Oil Red O lipid staining

The caloric value of various diets was assessed by feeding ~300 adult *P. pacificus* diets comprised of excess *E. coli* OP50, adult *C. elegans*, or larval *C. elegans* for 6 hours. As a control, *P. pacificus* was food-deprived for 6 hours. Oil Red O (ORO) lipid staining[57] was carried out as described by Escorcia et al.[58]. Stained *P. pacificus* animals were imaged on a Zeiss Axio Imager M2 microscope with a Hamamatsu colour CCD camera. Colour deconvolution[59] was done in ImageJ to separate ORO, background, and unstained body colours. ORO pixels were quantified as a percentage of worm body area.

Food switching

The food switching assay was adapted from the leaving assay described by Shtonda et al.[18]. Pairs of different food patches were placed 2 mm apart on a 35 mm NGM plate. *E. coli* OP50 spots were made by seeding 0.3 µl of liquid culture (OD_{600} = 0.4) and grown for 2 days. To produce *C. elegans* food patches, we used strains with locomotion phenotypes in order to restrict movement without use of anaesthetics, which would also affect *P. pacificus* and prevent free movement between food spots. Adult *C. elegans* spots consisted of ~20 animals with roller locomotion phenotype (IV95: *ueEx46 [gcy-7-sl2-mCherry; Punc-122::RFP]; gvIs246 [ida-1::GFP+ pRF4 rol-6(su1006)]*). Larval *C. elegans* spots consisted of ~500 animals with kinky locomotion phenotype (CB81: *unc-18(e81) X*). Food preference was assayed by placing a single adult *P. pacificus* in one food patch and checking 1 hour later to see if it had switched to the nearby

alternate food spot. Switching probability was calculated as the number of *P. pacificus* that switched divided by the total number of *P. pacificus* animals. Food preference was determined using the transitive property of inequalities.

Bacteria consumption and progeny proliferation

Initial bacterial supply was created by seeding 0.3 µl of OP50-GFP liquid culture (OD600=0.7) on 3% agar NGM 35 mm plates (with peptone omitted to minimize bacterial growth). Patches were allowed to saturate growth for 2 days. Initial bacterial levels were measured by imaging the OP50-GFP patches under fluorescence with consistent excitation and exposure parameters on a Zeiss Axio Zoom.V16 microscope and measuring GFP luminance. A single adult *P. pacificus* or adult *C. elegans* was placed by itself on a patch and imaged at 12 and 24 hours. GFP fluorescence, number of eggs, and number of hatched larvae were recorded.

Patch avoidance

To provide ample space for avoiding a patch, we used a larger arena (9.5 mm in diameter) with a 2 mm patch (medium density) in the centre. A single adult *C. elegans* and 3 adult *P. pacificus* were placed into the arena and recorded for 30 minutes at 0 and 6 hours. *C. elegans* exploration was scored as 'in' the patch when its body fully entered the patch, and 'out' of the patch when no part of its body touched the patch. *C. elegans* was considered on the 'edge' of the patch when only its mouth touched the patch.

Bite choice model

For a well-fed (with bacteria) *P. pacificus* individual presented with a particular *C. elegans* target and bacterial condition, bite value was estimated by calculating the expected utilities[7] of biting outcomes. We calculated the expected utility of each outcome,

$$EU_{i,j} = p_{i,j} \times v_{i,j} \quad (1)$$

where $p_{i,j}$ and $v_{i,j}$ are the probability and subjective value, respectively, of an outcome i (predatory, territorial) given an individual bite against a target j (larval *C. elegans*, adult *C. elegans*). Predatory outcomes are defined as feeding on the target, whereas territorial outcomes are defined as removing competitors from the bacterial territory.

First, we estimated $p_{i,j}$ using empirically obtained probabilities. For the probabilities associated with the predatory and territorial outcomes of a larva-targeted bite, $p_{P,L}$ and $p_{P,T}$, we used the empirically estimated probability that *P. pacificus* feeds on prey given a larva-targeted bite (Fig. 1e),

$$p_{P,L} = p_{T,L} = 0.8115 \quad (2)$$

We equated $p_{P,L}$ to $p_{T,L}$ since killing feeding on larvae simultaneously eliminates competitors. For the probability of a predatory outcome of an-adult targeted bite, $p_{T,A}$, we estimated the probability that an adult *C. elegans* exits a bacterial patch given a bite it receives while inside the patch (Fig. 1f).

$$p_{T,A} = 0.6507 \quad (3)$$

Since the objective probabilities values used for estimating $p_{P,L}$, $p_{T,L}$, and $p_{T,A}$ did not differ significantly across bacterial abundance (Extended Data. Fig. 2j,k), we assumed that $p_{P,L}$, $p_{T,L}$, and $p_{T,A}$ were constants and pooled data across bacterial conditions. Finally, for the probability of a predatory outcome of an-adult targeted bite, $p_{P,A}$, we measured the number of bites that a single *P. pacificus* inflicts on a single adult *C. elegans* in a bacteria-free arena (3.2 mm diameter)

until it successfully kills and feeds on the prey (Extended Data Fig. 2d). Since each successive bite may contribute cumulative harm in a way that kills *C. elegans* by attrition, the bite events are not independent of each other. Therefore the true $p_{P,A}$ should be a cumulative probability that is very low during the first bite and very high at ~25 bites. However, treating bites as cumulative or independent results in the same long-term incidence of killed prey, so we treated each bite as independent for simplicity of prediction,

$$p_{P,A} = \frac{1}{24.5} = 0.0408 \tag{4}$$

Next, we described outcome value as a function of bacterial abundance, $v(a)$. We divided bacteria abundance into three behaviorally defined subranges: negligible, scarce, and plentiful. The 'negligible' subranges encompassed the physical absence of bacteria, as well as bacterial abundance levels that are too small for *P. pacificus* to detect or care to exploit. We take the negligible subrange to be determined by sensory ability and internal state (hunger, satiety), and therefore consistent across outcome-target pairings when *P. pacificus* animals has been well-fed on OP50. The 'scarce' subrange included the minimum bacterial abundance that *P. pacificus* is willing to exploit, as well as other low levels of bacteria that induce *P.* pacificus to use biting as a means to secure additional food. Finally, the 'plentiful' subrange referred to excess bacterial abundance levels in which *P. pacificus* does not need to bite and focuses only on grazing on bacteria. Importantly, the scarce and plentiful subranges may vary depending on the outcome and target being considered.

Based on *P. pacificus*'s preference for bacteria food over prey (Fig. 1h), we generally defined predatory value functions as having a constant maximal value over the negligible

subrange where prey is the only acceptable food option, then monotonically decreasing over the scarce subrange, until it bottoms out to zero value over the plentiful range. We reasoned that predatory value over the negligible subrange should reflect the relative long-term net energy gain of eating prey when it is the only food option. Instead of calculating energy intake and dividing by food handling time, we approximate long-term net energy gain using ORO staining of fat stores (see Methods), a proxy indicator of excess energy intake (Fig. 1g). With excess food and assumed lack of satiety (OP50, the highest quality food, does not induce satiety[60]), we assumed that *P. pacificus* spent the entire time (6 hours) feeding and handling food (search time is assumed to be zero). Using the relative ORO-stained area in prey-fed *P. pacificus* compared to bacteria-fed *P. pacificus* (taken to be 1), we estimate predatory value of biting larval and adult targets over the negligible subrange,

$$v_{P,L}(a_{negligible}) = 0.4179 \tag{5}$$

$$v_{P,A}(a_{negligible}) = 0.5686 \tag{6}$$

For predatory value over the scarce subrange, we use the probability that *P. pacificus* switches from a prey patch to a bacterial patch (Fig. 1h) to linearly approximate how much prey *P. pacificus* foregoes with each increase in bacterial abundance,

$$v_{P,L}(a_{scarce}) = 0.4179 - 0.406a_{scarce} \tag{7}$$

$$v_{P,A}(a_{scarce}) = 0.5686 - 0.641a_{scarce} \tag{8}$$

Compared to predatory value functions, we set territorial value functions to be non-monotonic to reflect the multi-faceted dependence of bite value on both bacterial abundance and

on the more abstract property of bacterial territory. We reasoned that territorial value over the scarce subrange should be zero, since there is no bacteria territory present or worth defending. At the transition between negligible and scarce subranges, territorial value should jump suddenly to a maximal value, since this is where scarcity-induced competitive pressure is highest. Like predatory value functions, territorial value should also decrease monotonically over the scarce subrange. To estimate the maximal territorial value, we use the bacterial consumption rate of *C. elegans* relative to that of *P. pacificus* (Fig. 1i). Adult *C. elegans* consumes bacteria 1.5x faster than *P. pacificus*, but we found that the addition of L1 larvae (range 20-62) alongside an adult *C. elegans* did not increase bacterial consumption rate (Extended Data Fig. 3e). This finding differs considerably from previous reports that L1-L2 stage *C. elegans* consumes ~25% the rate of an adult *C. elegans*[61]. This discrepancy may be due to that study's use of liquid bacterial culture rather than a viscous patch, or due to our indirect measure of larval bacterial consumption (we did not measure larvae by themselves). To acquire a conservative estimate of larval bacterial consumption rate, we set adult *C. elegans* consumption to zero and assumed staggered hatching of larvae, and obtained a rate that is 1/20th the rate of adult *C. elegans*. To alleviate competitive pressure to defend territory, we reasoned that there should be additional bacterial allocated for *C. elegans* in addition to the amount that would be considered plentiful without *C. elegans* competition. To approximate this latter amount, we used the length of the scarce subrange for $v_{P,L}$. Altogether, we defined the territorial value over the scarce subrange for larval and adult *C. elegans,*

$$v_{T,L}(a_{scarce}) = 1.5 - \left(\frac{1.5}{1.0293(1+1.5)}\right) a_{scarce} = 1.5 - 0.589 a_{scarce} \quad (9)$$

$$v_{T,A}(a_{scarce}) = 0.05 - \left(\frac{0.05}{1.0293(1+0.05)}\right) a_{scarce} = 1.5 - 0.0463\, a_{scarce} \quad (10)$$

Finally, expected utility was calculated by multiplying the corresponding probability and value function for each target-outcome pair, and then comparing within-target to predict which outcome is more lucrative for a particular *C. elegans* target,

$$EU_{P,L} > EU_{P,A} \quad (11)$$

$$EU_{T,L} < EU_{T,A} \quad (12)$$

It is important to note that the purpose of this bite choice model is to predict specific inequalities within and across value functions, rather than to precisely predict *p(bite|encounter)* values.

Egg distribution

The egg distribution assay used the same behavioural setup as the patch avoidance assay. A variable 4-nematode mixture of adult *P. pacificus* and/or adult *C. elegans* were placed into the arena and removed 7 hours later. A C. elegans strain with integrated GFP reporter that expresses in eggs (CX7389: *kyIs392 [Pstr-2::GFP::rab-3; Pttx-3::lin-10::dsRed; Pelt-2::GFP]*) was used to visually distinguish *C. elegans* eggs from non-fluorescent *P. pacificus* eggs. Egg plates were incubated at RT for one hour and then at 4°C for 2 days to allow GFP expression to increase while preventing hatching. Arenas were then imaged under bright-field and fluorescence microscopy using a Zeiss Axio Zoom.V16 microscope. The distances of eggs from the centre of the patch were measured using MATLAB. Probability density functions in Figure 2d were fitted onto histograms of eggs distances.

Larval patch-finding

The larval patch-finding assay used the same behavioural setup as the patch avoidance and egg distribution assay. Mature CX7389 eggs were transferred from a bacteria-depleted plate to one side of a clean 3% agar NGM plate. Ten newly hatched L1 larvae found on the opposite side of the plate were transferred to a specific radius from the centre of the patch. Plates were checked on a Zeiss Axio Zoom.V16 microscope 36 hours later for the presence of fluorescent larvae inside the patch.

Biting incentive

Biting incentive was measured using the same behavioural setup for assaying the immediate consequences of biting (arena 3.2 mm in diameter, 30 minutes, 1 adult *P. pacificus* with ~100 larval *C. elegans* or 1 adult C. elegans). Our criteria for determining encounters and bites were slightly modified from those used by Serobyan et al.[16] and Wilecki et al.[17]. Bites were scored the same way as for measuring capture success. Encounters were counted when: 1) the *P. pacificus* mouth fully contacted the *C. elegans* body, and 2) *P. pacificus* interrupted it normal locomotion by slowing down or contorting its head toward *C. elegans*, thereby positively indicating detection of *C. elegans*. Biting incentive was calculated by dividing the number of bites by the number of encounters, $p(bite|encounter)$.

Amphid neuron ablation

DiO staining of amphid neurons was adapted from published staining of *P. pacificus*[62]. Larval J2 *P. pacificus* were stained for 2 hours on a nutator in a solution of 15 ng/ml Fast DiO (ThermoFisher D3898) and then de-stained on an empty NGM plate for 1 hour. A 3% agar plug was used to gently transfer stained J2 animals onto a 2% agarose pad (melted in M9) with 20

mM sodium azide paralytic. Pairs of amphid neurons were ablated using an Andor Micropoint focused laser microbeam system. Cell identification was based on the identities described by Hong et al.[63]. Cell death was confirmed by identifying a morphological change within the cell, and by re-staining after behaviour was recorded. Each ablated J2 was transferred onto its own bacterial patch to recover before being used 2 days later in a behavioural assay.

Search speed

Since the *P. pacificus* mouth is engaged in both feeding on bacteria and biting *C. elegans*, we tracked mouth location instead of the body's centre of mass. Mouth location on a bacterial patch (medium density, 1 mm in diameter) was manually tracked using MATLAB. To focus on deliberate on-patch exploration patterns, we restricted analysis to the longest continuous video segment (≥ 10 minutes of a 30-minute recording) during which *P. pacificus* did not leave the patch. To measure total movement, we calculated translational speed as the sum of the Euclidean distances between each mouth location, divided by total time. To measure patrolling around the circular patch boundary, we first measured the widest arc of the patch circumference that *P. pacificus* traversed (excluding back-and-forth movements that do not contribute to forward progress) in between changing directions (clockwise ↔ counterclockwise) or traveling along a chord to another location on the patch circumference. Then, we summed all arcs (radians) and divided by total time to arrive at what we call patrol speed. The ratio of patrol speed to translational speed was used to compare differences in how much movement is dedicated to patrolling. This speed ratio is especially relevant when looking at speed when larval *C. elegans* are present, since *P. pacificus* has bouts of stationary feeding on prey.

Drug screen and follow-up treatments

The drug screen was conducted using a subset of the Library of Pharmacologically Active Compounds (LOPAC^{1280}). Only drugs with documented biological action on neurotransmission were used, a total of 680 compounds. In all rounds of the drug screen, *P. pacificus* was treated and effects were indirectly assayed by observing *C. elegans* egg distribution. All egg distribution assays included 3 adult *P. pacificus* and 1 adult *C. elegans.* To expediently test 680 compounds in the first round of the drug screen, *C. elegans* was treated alongside *P. pacificus* during the 7-hour egg distribution assay to avoid a separate treatment phase. Two µl of 1 mM of each compound was directly placed onto the patch of the egg distribution arena and allowed to dry before placing animals. The top 135 compounds with the highest mean off-patch egg distance in the first round were selected to be tested again in second set of egg distribution assays. For second and third rounds of the drug screen, *P. pacificus* animals were treated in the same way but in a separate 2-hour phase prior to the egg distribution assay, and candidates were selected based on both the percentage of eggs laid off-patch and the mean off-patch egg distance. From the 135 drugs tested in the second round, 9 were selected to continue to the third round, which collected multiple data points for each of the 9 candidates.

Treatment of *P. pacificus* for behavioural recordings of biting was done using similar patch treatments. The patch used for treatment was formed by seeding 0.5 µl of liquid *E. coli* OP50 cultures (OD_{600} = 0.4) on a 35 mm NGM plate and growing for 2 days. Two µl of a stock drug solution (5 mM amisulpride, 10 mM sumanirole maleate, 100 mM octopamine, 100 mM epinastine) was dispensed onto the patch, 1 µl at a time and allowed to dry in between. As soon as the patch was visibly dry, *P. pacificus* young adults were placed on the treated patch for 2 hours before use in behavioural assays.

Methods References

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Author contributions

All experiments were conceived of and performed by K.T.Q. All analysis was done by K.T.Q. The manuscript was written by K.T.Q. and S.H.C.

Competing interests

Authors declare no competing interests.

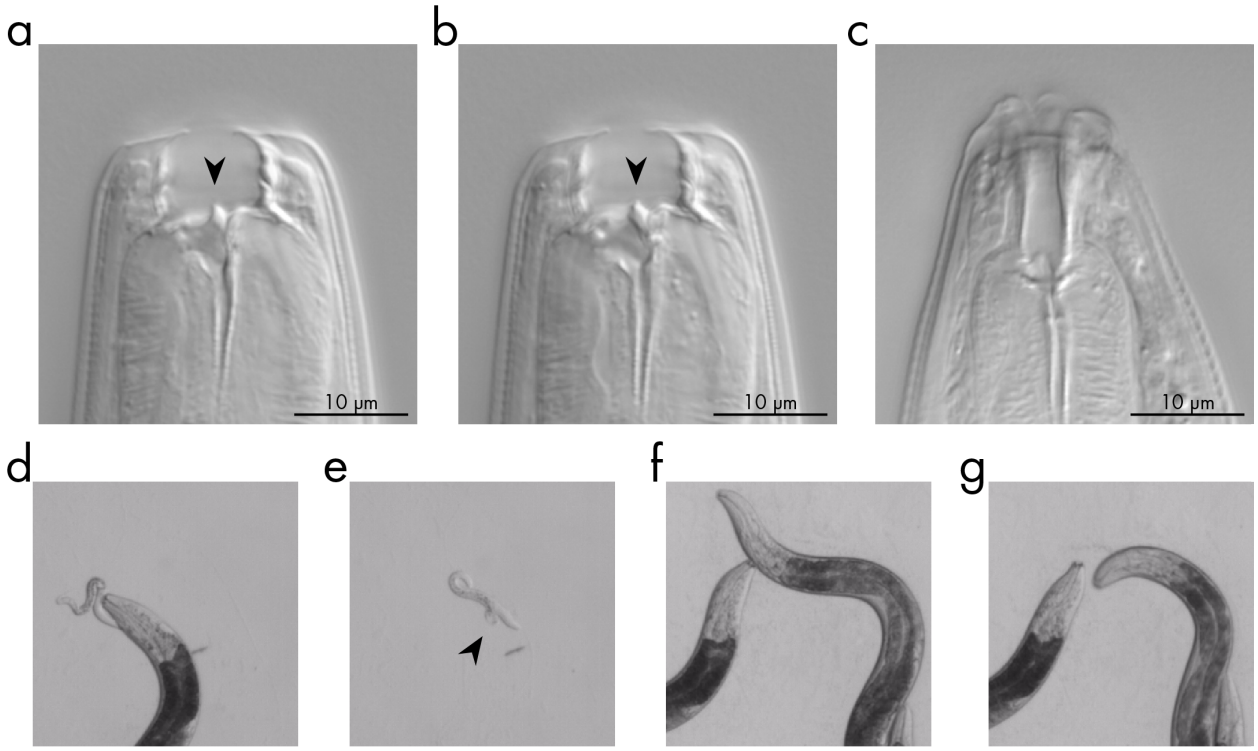
Additional information

Supplementary Information is available for this paper.

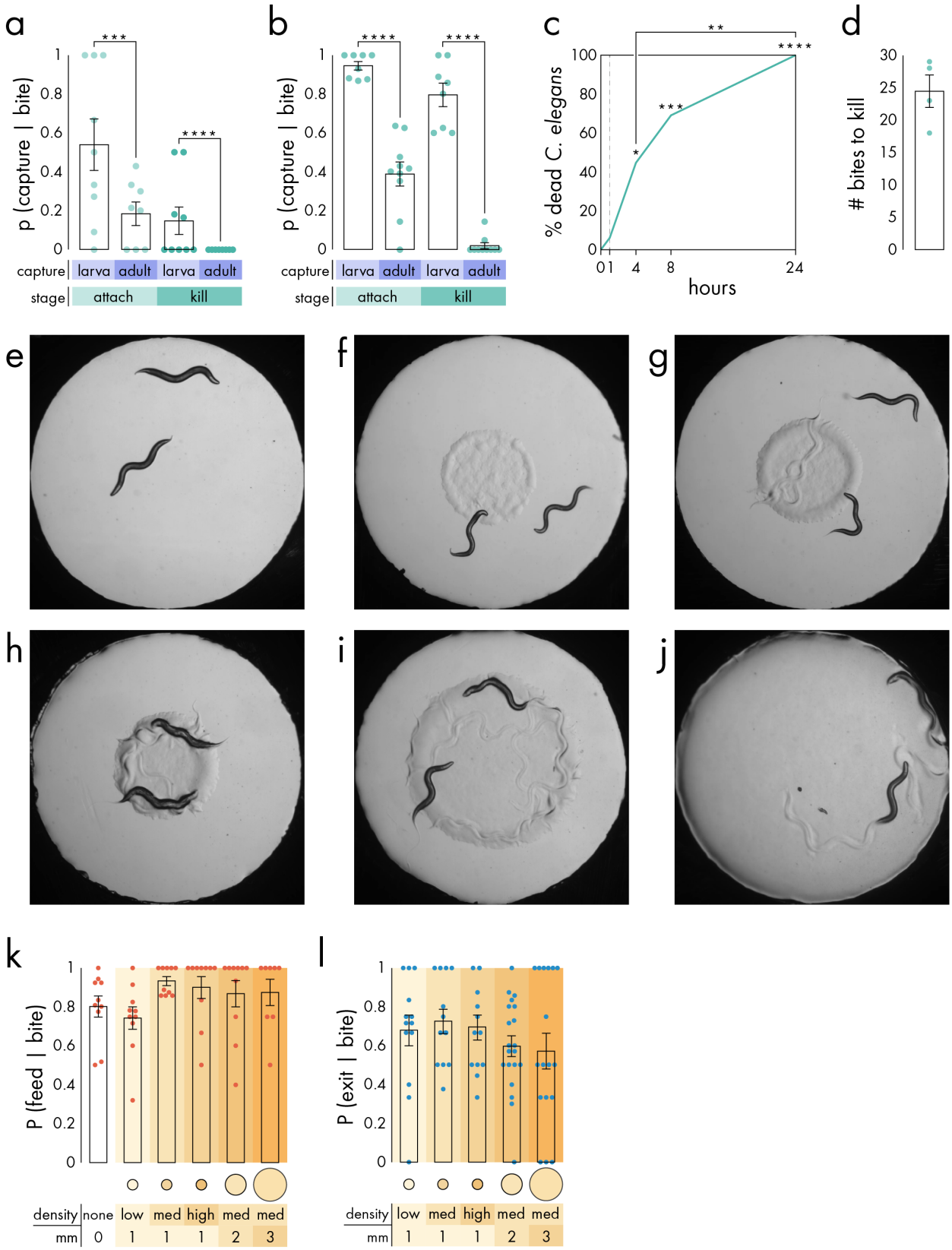
Correspondence and requests for materials should be addressed to S.H.C. (schalasani@salk.edu).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.



Extended Data Fig. 1 | a, Ventral and **b,** dorsal tooth of eurystomatous *P. pacificus* adult. **c,** *C. elegans* mouth (no teeth). **d,** Biting of larval (L1) *C. elegans*. **e,** Leakage of pseudocoelomic fluid after lethal biting of larval (L1) *C. elegans*. **f,** Biting of adult *C. elegans*. **g,** Adult *C. elegans* escaping without injury from a bite.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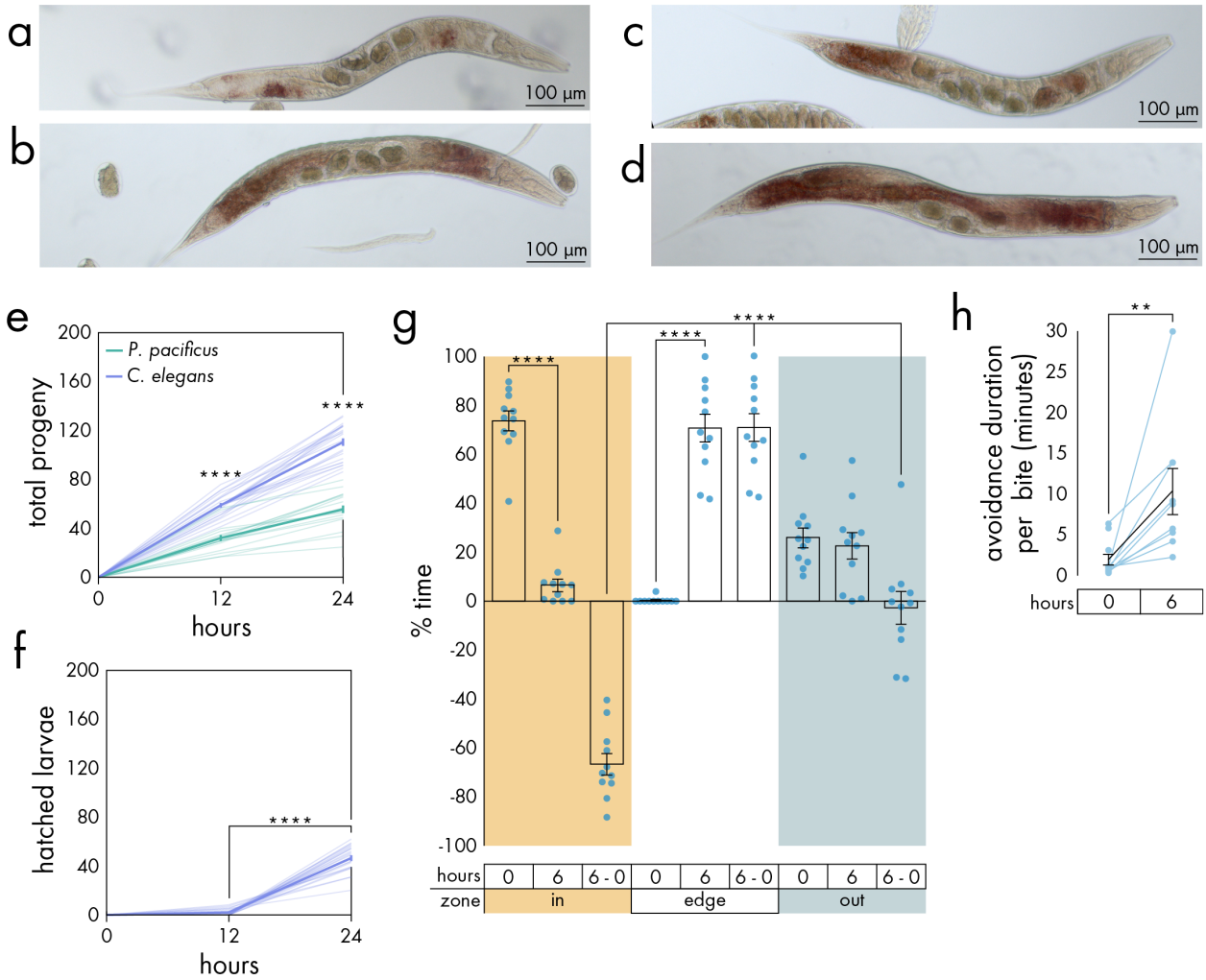
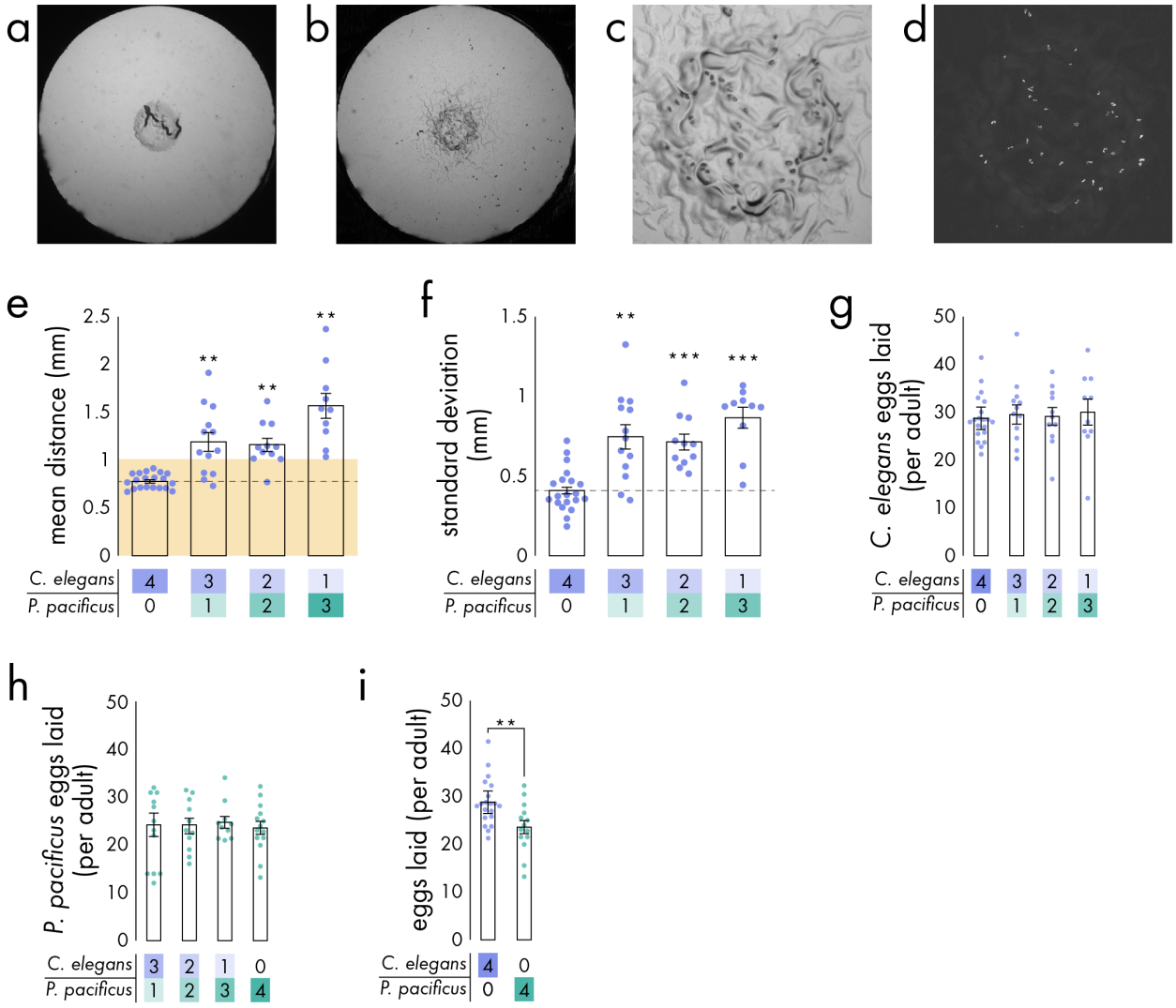
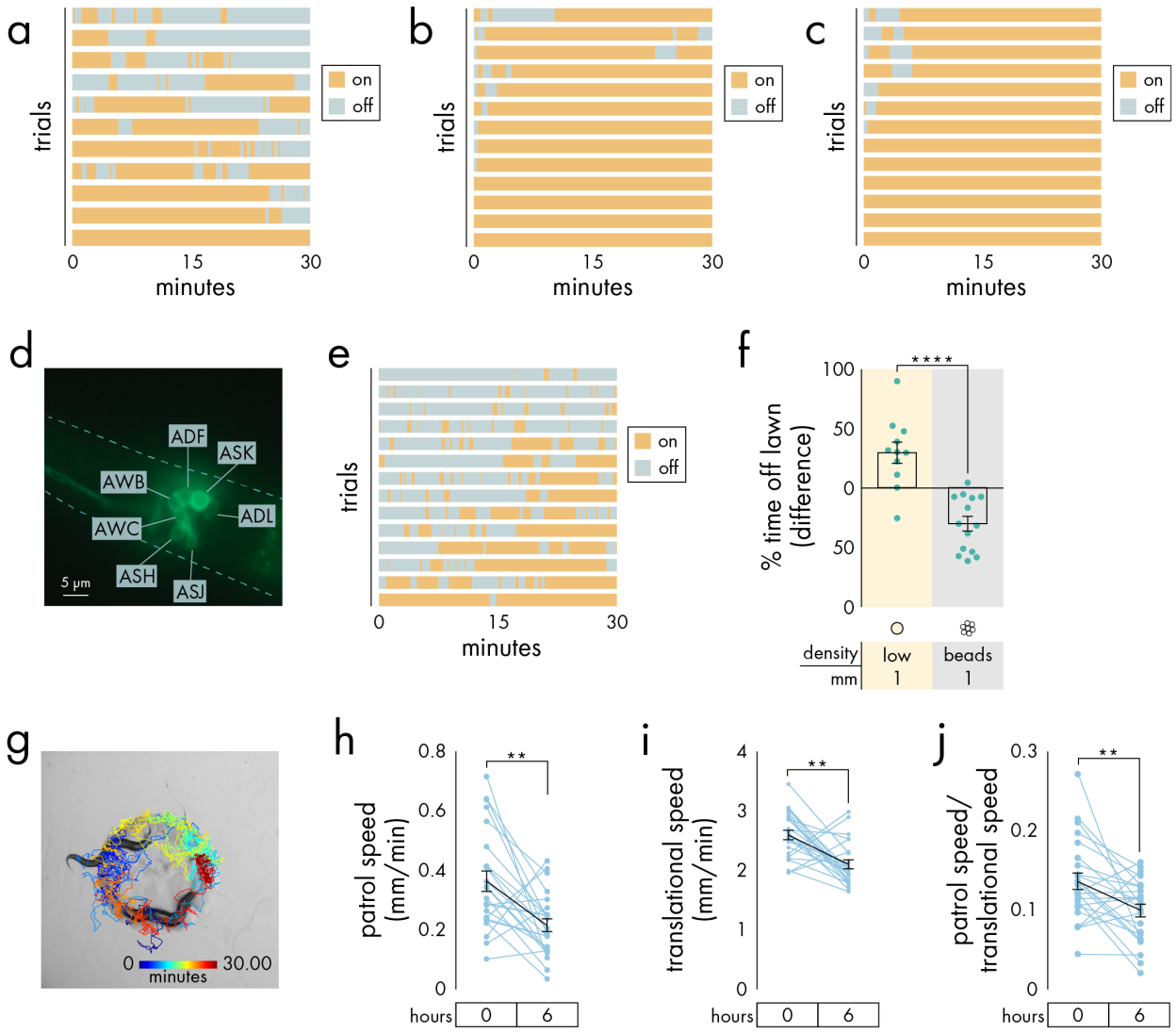
Extended Data Fig. 2 | a, Capture success of PS312 *P. pacificus* (logistic regression, $n_{P.pacificus}$ = 8-9). **b,** Capture success of RS5194 *P. pacificus* (logistic regression, $n_{P.pacificus}$ = 8-12). **c,** Hours of biting required to kill a single adult *C. elegans* (Fisher's exact test with Benjamini-Hochberg correction, $n_{P.pacificus}$ = 16). **d**, Number of bites required to kill a single adult *C. elegans*. **e,** Arena with no bacteria. **f-j,** *E. coli* OP50 bacterial patches of different densities (see Methods) and diameters. **f,** Low-density, 1 mm. **g,** Medium-density, 1 mm. **h,** High-density, 1mm. **i,** Medium-density, 2 mm. **j,** Medium-density, 3 mm. **k,** Probability of feeding given biting of larval *C. elegans*, across bacterial conditions (logistic regression, $n_{P.pacificus}$ = 9-10). **l**, Probability that adult *C. elegans* exits a bacterial patch after being bitten on different types of patches (logistic regression, $n_{P.pacificus}$ = 13-20). All error bars are mean ± 1 s.e.m.



Extended Data Fig. 3 | a-d, Oil Red O staining of *P. pacificus* after 6 hours of feeding on **a,** nothing, **b,** larval *C. elegans*, **c,** adult *C. elegans*, and **d,** *E. coli* OP50 bacteria. **e,** Total progeny and **f,** hatched larvae produced by a single adult *P. pacificus* or *C. elegans* (negative binomial regression, n_{adult} = 16-23). **g,** *C. elegans* residence time in, out, and at the edge of a bacterial patch when *P. pacificus* was present (Welch's t-test with Benjamini-Hochberg correction, $n_{C.elegans}$ = 11), and differences between time points (Tukey's test, $n_{C.elegans}$ = 11). **h,** *C. elegans* patch avoidance time per bite (Wilcoxon's signed rank test, $n_{C.elegans}$ = 9-11). All error bars are mean ± 1 s.e.m.



Extended Data Fig. 4 | a-d, Egg distribution assay at **a,** 0 hours, **b,** 7 hours, **c,** zoomed-in patch, **d,** zoomed in patch, with fluorescent *C. elegans* eggs. **e,** Mean distance from the patch centre and **f,** standard deviation of *C. elegans* eggs (Dunnett's T3 test, n_{trials} = 10-20). **g,** Total eggs laid per *C. elegans* adult across mixed-species groups (negative binomial regression, n_{trials} = 10-20). **h,** Total eggs laid per *P. pacificus* adult across mixed-species groups (negative binomial regression, n_{trials} = 10-20). **i,** Total eggs laid per *P. pacificus* adult across mixed-species groups (negative binomial regression, n_{trials} = 14-20). All error bars are mean ± 1 s.e.m.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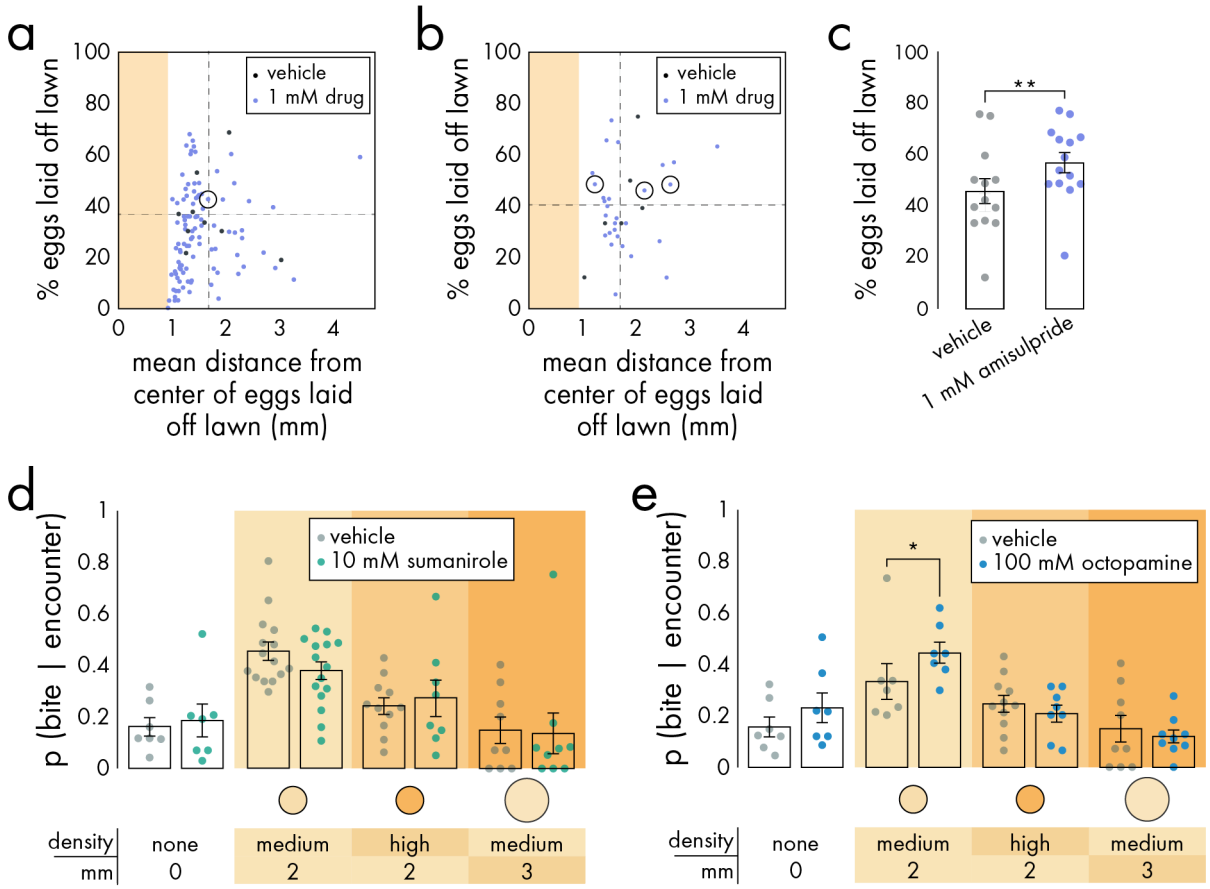
Extended Data Fig. 5 | a-c, Timecourse of *P. pacificus* on/off a **a,** low-density bacterial patch (1 mm), **b,** medium-density patch (1mm) and **c,** high-density patch (1 mm). **d,** *P. pacificus* larva (J2 stage, anterior on left) with ciliated amphid neurons stained with DiO. **e,** Timecourse of *P. pacificus* on/off a bead patch (1mm). **f,** Difference between 0-15 min and 25-30 min in patch residence time (t-test, $n_{P.pacificus}$ = 11-14). **g,** *P. pacificus* tracks on a patch with another *P. pacificus.* **h,** Long-term change in patrol speed (paired t-test, $n_{P.pacificus}$ = 24). **i**, Long-term change in translational speed (Wilcoxon's signed rank test, $n_{P.pacificus}$ = 24). **j**, Long-term change in speed ratio (paired t-test, $n_{P.pacificus}$ = 24). All error bars are mean ± 1 s.e.m.



Extended Data Fig. 6 | a, 2nd, and **b,** 3rd rounds of drug screen (see Methods). Black circles indicate amisulpride. **c,** Effect of amisulpride on percentage of eggs laid off patch (logistic regression, $n_{C.elegans}$ = 13-14). **d,** Effect of sumanirole on *p(bite|encounter)* (logistic regression, n_{trials} = 7-15). **e,** Effect of octopamine on *p(bite|encounter)* (logistic regression, n_{trials} = 7-11).

Legends for Supplementary Videos

Video 1 | *P. pacificus* bites a young adult *C. elegans* in the absence of bacteria, followed by *C. elegans* escaping from the bite

Video 2 | *P. pacificus* bites, kills, and feeds on a *C. elegans* larva (L1).

Video 3 | *P. pacificus* decides to stay on a 1 mm, low-density bacteria patch and bites adult *C. elegans*. Adult *C. elegans* exits the patch after being bitten.

Video 4 | *P. pacificus* encounters and decides to leave a 1 mm, low-density bacteria patch.

Video 5 | *P. pacificus* enters and patrols around the boundary of a 1 mm, medium-density bacteria patch.

Video 6 | *P. pacificus* bites adult *C. elegans* on a bacteria patch.