Carp Fever Revisited: The Evolution of Amino Acids in Carp Fishing

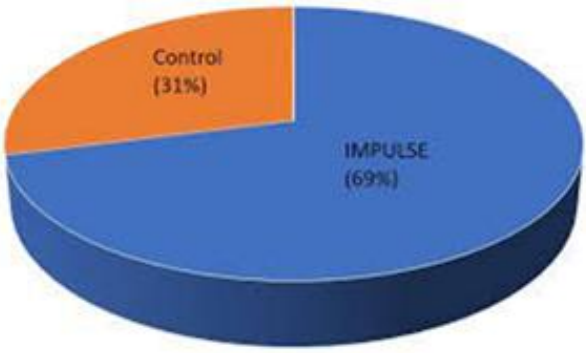
Part 1: Carp Fever's Unanswered Questions

Hungry Like the Wolf

If, like me, you're also getting a little long in the tooth, you'll likely have a ragged, ear marked copy of *Carp Fever* knocking around somewhere. For those who don't involuntarily burst into song every time Duran Duran's *Hungry like the Wolf* comes on the radio, Kevin Maddocks' *Carp Fever* was also, in many ways, truly groundbreaking and helped define the 1980s - carp fishing's most formative decade. Specifically, not only did *Carp Fever* introduce anglers to the hair rig, but, perhaps more significantly, this landmark publication also featured the results of tank tests focused on ranking the feed inducing properties of individual amino acids.

Now, better than 40 years after the publication of Maddock's original work, more recent academic studies have not only validated Kevin's findings, but have also allowed for a deeper understanding of the underlying physiology of how amino acids (AAs) generate an involuntary feeding response in carp. These advancements have allowed for the development of dedicated Asian Carp attractants by a federal research agency in the United States (USGS), as well as, more importantly for carp anglers, a new class of feeding stimulants that, when added to anglers baits, have been scientifically proven to better than double catch rates. While relevant details from this latter project are further discussed throughout this article, anglers are encouraged to review copies of the original research dissertations, conducted at Sparsholt College, at either the AminoTec.com or BioSourcebaits.com websites.

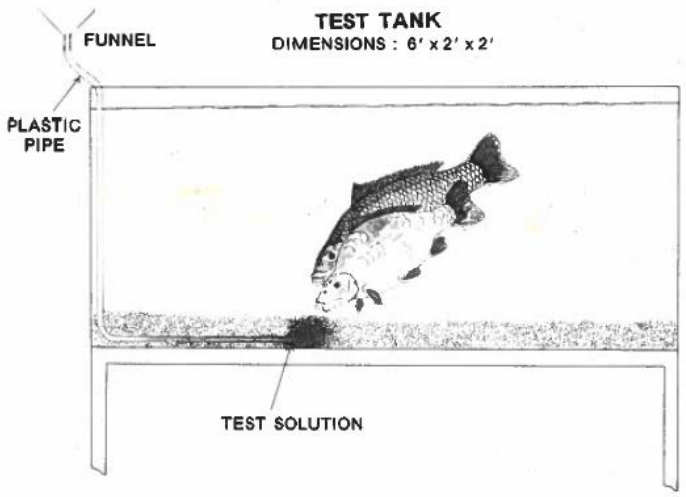
What follows is a discussion of how the original work featured in *Carp Fever* initiated the evolution, and ultimately the creation, of this radical new breed of amino acid-based fish feeding stimulants. Particular attention is paid to how lessons learnt, through a rigorous science focused reinterpretation of Maddocks' results, as well as the solving of some of *Carp Fever's* unanswered questions have, ultimately, allowed for the 'cracking of the amino acid code' – i.e. the development of a practical model of AA induced stimulation. Once grasped, anglers can readily apply this knowledge to their own bait manufacture and preparation methods and, most importantly, enjoy the resultant benefits of significantly enhanced catch rates.



Above: Summary of Results from the second of two Sparsholt studies showing Impulse dosed v undosed baits catch rates

Left: USGS Researcher with an Asian Carp captured during testing of an Impulse™ prototype

The Original Work



Carp Fever was truly ahead of it's time in terms of how it described the response of carp to amino acids. Briefly, AA solutions were leaked into the gravel covering the bottom of a large tank containing several mature carp, with these fishes' feeding responses then observed. This simple methodology yeilded *Carp Fever's* signiture 'league table' of AA potencies which, in turn, confirmed Lysine and Valine to be the most effective AAs at generating a feeding response.

Excellent Reaction ('Best fit')	Good Reaction ('Good fit')	Poor Reaction ('Poor fit')	No Reaction ('No fit')
Valine (VAL) Lysine (LYS)	Asparagine (ASN) Phenylalanine (PHE) Histidine (HIS) Glycine (GLY) Isoleucine (ISO)	Alanine (ALA) Proline (PRO) Cystine (CYS) Betaine*	Argenine (ARG) Glutamine (GLN) Threonine (THR) Leucine (LEU) Tyrosine (TYR) Aspartic acid (ASP) Glutamic acid (GLU)

Sidechains: Red = acidic Blue = basic Purple = neutral Green = polar. *Betaine is a modified AA

Maddocks' results have certainly stood the test of time, with a number of more recent academic studies, using a variety of more modern techniques, returning essentially identical results to those first described in *Carp Fever*. Indeed, our current 'state of the art' AA potency table (shown above) was constructed through combining the results of all known studies and is, for all intents and purposes, essentially identical to that first recorded by Kevin. For me, these findings formed the foundation of what has become a life-long obsession with amino acid induced feeding behavior. Briefly, I have been conducting serious academic research into the topic for almost twenty years, with this work leading directly to the development of a range of species-specific fish attractants and feeding stimulants through a collaborative research project with the United States Geological Survey (USGS) and, ultimately, to the founding of a pair of businesses focused on the development and sales of angling products (AminoTec R&D and BioSource Baits). The effectiveness of these new discoveries, most notably Impulse™, are rooted in theory that continues to evolve from *Carp Fever*'s foundational work. The mission of this article is to bring anglers up to date with this journey and in doing so, as mentioned above, provide an understanding of how amino acids, when used correctly, can best be utilized with regard to the creation and implementation of new, more effective baits. Our first step in this process begins with a brief review of a few fundamentals relating to how fish detect, and are initiated into feeding, through AA induced processes.

Amino Acids and Their Receptor Sites

There are 20 naturally occurring amino acids found in nature, with chains of these AAs making up the proteins found in all living things. In many ways, AAs may be considered nature's 'Legos', with most of the body's structures constructed from longer chains of these simple building blocks. One fundamentally important observation is that each of the 20 natural AAs belong to one of four specific classes – Acidic, Basic, Neutral or Polar. This fact has relevance for carp anglers, as *Carp Fever*, in addition to more recent studies (see above league table), clearly showed that AAs from the Basic (LYS) and Neutral (VAL) classes are most stimulatory to carp,

while Acidic AAs (ASP, GLU) are not. From a biochemist's point of view, this detail clearly implies that carp possess a pair of olfactory (smell) chemoreceptors dedicated the recognition of Basic and Neutral AAs – a fact that now underpins our bivalent model of AA induced stimulation; while, for anglers incorporating single AAs into their own baits, their choices should be restricted to AAs from either the Basic or Neutral classes. Having said that, simply plopping a teaspoon of Lysine or Valine into a base mix will NOT provide the desired effects – as is described more fully below, **anglers need to exercise extreme caution when utilizing single AAs as feeding stimulants / attractants, as inappropriate application will almost certainly *not* yield the desired results**.

Protein Breakdown, Amino Acid Profiles and Hydrolysates

Proteins within all organisms, including fish, and even humans, are continually being broken down and replaced, with any superfluous AAs simply being excreted. Not so sure? Fingerprints left on the pages of a magazine or keyboard can readily be identified by a ninhydrin test - a process that stains AAs blue (so rendering them readily viewable to 'plod'). For aquatic creatures, their respective AAs dissolve directly into the surrounding water. It is these solvated AAs that are ultimately detected by fish, allowing them to home in on a potential meal. As a consequence, the chemosensory apparatus of fish, and even far more primitive organisms, such as flatworms and even motile bacteria, have become programed to first detect, and then swim towards, an increasing concentration of amino acids. **This is an important fact that anglers must acknowledge – it is the trail of dissolved, aka 'free', AAs within a body of water that initially attract and then, ultimately, invoke a feeding response in fish**.

The 'plume' of free amino acids generated by any aquatic organism takes the form of a characteristic AA profile. Briefly, as shown by the following table, any such amino acid 'soup' will contain all 20 natural AAs, with each AA being present at a specific, fixed level. Each foodstuff will generate a unique profile, with proteins derived from meat sources tending to be richer in Basic AAs, notably Lysine, while Neutral AAs, such as Valine, will tend to dominate the profiles of vegetable proteins. Interestingly, hydrolysates, derived through either the chemical hydrolysis or natural digestion of proteins, are essentially comprised of the same free AAs, in identical relative amounts, to those found in the food's corresponding AA profile. While these predigested protein sources certainly make great additions to any base mix (greatly enhancing its nutritional profile), caution should be exercised when using hydrolysates as a glug or bait soak. Briefly, as is discussed fully below, the stimulatory effects of dissolved AAs become 'cancelled' at higher concentrations when AAs from dissimilar classes are combined. This effect was first observed by Maddocks and reported in *Carp Fever*: "one conclusive finding was that those acids which were best on their own, proved to be ineffective when used in combination". **Thus, in order to mimic the chemosensory properties of naturally excreted AA blends, liquid hydrolysate glugs and dips should be used sparingly. If a strong feeding response is desired, a dedicated feeding stimulant, such as Impulse™, should be preferentially employed.**

Amino Acid	Proteins and Their Amino Acid Composition (%)							
	egg white	tuna	beef	chicken	whey	casein	soya	yeast
alanine	6.6	6.0	6.1	5.5	5.2	2.9	4.2	8.3
arginine	5.6	6.0	6.5	6.0	2.5	3.7	7.5	6.5
aspartic acid	8.9	10.2	9.1	8.9	10.9	6.6	11.5	9.8
cystine	2.5	1.1	1.3	1.3	2.2	0.3	1.3	1.4
glutamic acid	13.5	14.9	15.0	15.0	16.8	21.5	19.0	13.5
glycine	3.6	4.8	6.1	4.9	2.2	2.1	4.1	4.8
histidine	2.2	2.9	3.2	3.1	2.0	3.0	2.6	2.6
isoleucine	6.0	4.6	4.5	5.3	6.0	5.1	4.8	5.0
leucine	8.5	8.1	8.0	7.5	9.5	9.0	8.1	7.1
lysine	6.2	**9.2**	**8.4**	**8.5**	8.8	3.8	6.2	6.9
methionine	3.6	3.0	2.6	2.8	1.9	2.7	1.3	1.5
phenylalanine	6.0	3.9	3.9	4.0	2.3	5.1	5.2	4.7
proline	3.8	3.5	4.8	4.1	6.6	10.7	5.1	4.0
serine	7.3	4.0	3.9	3.4	5.4	5.6	5.2	5.1
threonine	4.4	4.4	4.0	4.2	6.9	4.3	3.8	5.8
tryptophan	1.4	1.1	0.7	1.2	2.2	1.3	1.3	1.6
tyrosine	2.7	3.4	3.2	3.4	2.7	5.6	3.8	5.0
valine	7.0	**5.2**	**5.0**	**5.0**	6.0	6.6	5.0	6.2

AA Classes: Red = acidic Blue = basic Purple = neutral Green = polar.

You Said What Now?

Let's recap what we've learnt so far. First, *Carp Fever* clearly showed that carp are stimulated to feed by solutions of single amino acids, with AAs from either the Basic or Neutral classes proving to be the most effective. Second, Maddocks also proved that when AAs from dissimilar classes are mixed, their stimulatory effects are 'cancelled'. *You said what now???* Let that sink in for a moment – "amino acids which were best on their own, proved to be ineffective when used in combination". How does that even make sense? How can mixing Lysine and Valine, the two most stimulatory AAs, generate a blend that is essentially useless as a feeding stimulant? Not wishing to sound overly dramatic, but this single sentence 'lit a fire' under this eager, wide-eyed teenager back in the 1980s, literally launching a lifelong crusade into answering this most significant of *Carp Fever's* unanswered questions. What follows is a summary of how this goal was achieved, resulting in the creation of both our Bivalent model of chemosensory stimulation and, most importantly, Impulse™ – the world's first dual amino acid fish feeding stimulant scientifically proven to better than double anglers' catch rates.

Part 2: Cracking the Amino Acid Code

Zero - Hero - Zero and The Goldilocks Zone

I was hired as a Chemistry Professor in 2003, a position which I still hold today. One of the great things about academia is the freedom to explore novel problems through research – bingo! At last, I now had the time, opportunity and resources to dive into addressing the "you said what now?" amino acid conundrum posed by *Carp Fever*. The first step in this journey was (not so) simple – confirming Maddocks' single amino acid results.

Reproducing *Carp Fever*'s results initially proved to be both challenging and highly frustrating. The problems were eventually traced to multiple sources, with the most significant of which being what we now 'fondly' refer to as the 'zero - hero - zero' effect. Simply put, dissolved single amino acids have an extremely low strength (concentration) below which no attraction is observed ('zero'). After a critical threshold concentration is attained, attraction, followed by involuntary feeding, occurs over a *very narrow* 'goldilocks' range of concentration ('hero'), ultimately ceasing completely as AA concentration further increases ('zero'). For the 'bait heads' out there, we have determined Lysine to have a threshold of attraction at ~0.050 ppm, with optimal feeding stimulation occurring at ~0.20 ppm, with negligible stimulatory effects observed above ~2.0 ppm. In practical terms, this equates to needing to maintain the equivalent of approximately 0.0031 grains (~0.20 mg) of dissolved AA around the baited area at all times to establish the desired 'Goldilocks zone' of concentration required to generate an optimal feeding response – a feat that, I think we'd all agree, is clearly beyond the expertise of most anglers!

The 'zero - hero - zero' effect underpins both the recommendation presented in the previous section, with regard to exercising extreme caution when using single AAs, while also allowing for a rationalization of the hit and miss results of anglers who have attempted to utilize single amino acids as attractants in the past. Specifically, this effect was clearly witnessed by Ken Townley and reported in his book *The Beekay Guide to Carp Baits*, where it is confirmed that: "(betaine) is one of those products that needs to be used carefully. Put too much in and you'll spoil the effect but get the level just right and you'll have them *crawling up the rods"*.

So, at this point you are likely asking yourself an important question – if the 'Goldilocks zone' is so narrow and hard to achieve, then how on earth was Maddocks able to pull off those experiments from *Carp Fever*? Great question! Simply put, because Keven introduced his AA solutions through a pipe to a location *under* the gravel in his tank, the ambient concentration of AAs, resulting from their perfusion into the tank, as a direct consequence, must begin at zero before *slowly* ramping through the 'Goldilocks zone'. Kevin's observations most likely mirrored the effects of this process, so allowing for the creation of his signature league table of AA potencies. Importantly, through using a modified *Carp Fever* style approach, we have been able to begin the process of quantifying the table of AA potencies. Briefly, we have confirmed the simple assumption that less potent AAs need to be introduced at proportionally higher levels

than their more potent counterparts in order to invoke similar stimulatory effects. Specifically, we have so far been able to show that Lysine is indeed the most potent AA, with Valine and Alanine, respectively, being ~2 and ~5 times less potent than Lysine. Thus, it would appear as though the difference between 'Excellent' and 'Poor' reactions span approximately one order of magnitude, or, in other words, you would need approximately 10 times more Betaine (poor column) than Lysine (excellent column) to generate the same effective response. **This observation in itself is of great interest to anglers, as it infers that the specific choice of (Basic or Neutral) stimulatory AA used is rendered largely irrelevant so long, as is discussed in further detail below, a *very precise* dosage is *strictly* employed**.

Back To the Drawing Board

As indicated above, the extremely narrow window of concentration, i.e. 'Goldilocks zone', associated with single amino acid stimulation severely hinders their use as viable fish feeding stimulants. However, while this objective is indeed very difficult to achieve, it is not impossible…

In 2006/7, along with my good friend Trevor Burgess, we began testing the viability of Lysine as a feeding stimulant for carp. As two Brits living in the USA, we'd get a few odd looks and occasional comments from baffled 'civilians' as we fished side by side with our 13-meter carbon poles. This 'Euro-centric' methodology was essential however, as *only* through feeding strictly regulated volumes of feed pellets (via a pole cup), which were, in turn, wetted using a Lysine solution of a precisely known concentration, at defined time intervals, were we able to determine the crucial set of parameters needed to generate an enhanced feeding response. Briefly, over *many, many* trials we were able to determine what we now refer to as the 'rule of 15s' – we simply potted in ~15 mL of dosed feed pellets every ~15 mins. Our results, shockingly, wildly exceeded our expectations, with catch rate differentials of ~2:1 eventually being consistently recorded for dosed vs undosed baits. Additionally, our results were confirmed by our team of UK based match fishing testers, who began to steadily collect increasing numbers of brown envelopes, as well as through our first collaboration with Sparsholt College, in 2010, where Thomas Meier's research dissertation "*The Effect of BioSource Feed Stimulant on the Bite Numbers in a Controlled Fishing Environment*" concluded there to be "a significant difference between bite rates", and that there was "sufficient evidence and statistical analysis showing there to be a significant difference between stimulated feed to that of a control".

With the effectiveness of single AA stimulants now proven, we took the plunge and launched our first commercial product, BioSource™, in 2011. While BioSource™ proved to be an excellent match fishing product, lending itself perfectly to a 'little and often' style feeding strategy, it, unfortunately, underperformed with classic 'big carp' applications, such as spodding, where greater volumes of bait are introduced. In hindsight, it now seems clear that BioSource™ was indeed emulating the effects witnessed by Maddocks in *Carp Fever*. Briefly, a 'little and often' strategy, as epitomized by the 'rule of 15s', similarly generated a slowly ramping concentration of AAs each time a small portion of feed was introduced. Significantly, however, the gradual

decline of stimulatory response seen at higher AA concentrations ('Zero - Hero – Zero'), as witnessed both in the lab and when spodding, was generally not encountered when implementing a 'little and often' approach. The underlying reasons why this strategy does not over stimulate the fish, i.e. through not generating excessive AA levels, can be easily rationalized - small volumes of bait, introduced at regular intervals, typically cause the fish to feed competitively, meaning that each portion of bait would likely be consumed before it's full AA payload was fully released into the water. This effect was also likely enhanced by the bait immediately beginning to release its AAs upon introduction to the swim, so enhancing the competitive feeding response via the initiation of stimulation. These events also mesh well with the match fisherman's natural tendencies to 'feed to response', as a decline in bite frequency is correlated with a decline in AA concentration in the swim as the feed is 'mopped up', so prompting the angler to refeed and restart the cycle.

While BioSource™ certainly proved that creating a viable amino acid feeding stimulant was indeed possible, the fact that this product was only effective when used in conjunction with a strict 'little and often' feeding strategy rendered it unsuitable for use with a variety of other popular baiting applications, such as spodding. Thus, in 2012 we went back to the drawing board, with the ultimate goal of developing a next generation embodiment capable of providing an optimal level of feeding response when used with a range of popular baits and applications.

Unfortunately, getting to grips with this lofty objective demanded a critical change in thinking – we needed to develop a molecular scale model of exactly how the carp's chemoreceptors process AA stimulus, as only such a model would, firstly, reveal if our goal was even achievable, and, secondly, provide the essential route map to its eventual solution. Don't worry, as I like to remind my students, nature always provides simple answers to our often overly complex human questions. So, if you've ever played with Legos, used a key to open a door, or played a game of draughts (checkers US) you already have a good handle on the necessary commonsense concepts needed. Right then, if you are ready, let's 'pop the hood' and see what really makes carp tick…

Popping the Hood on Chemosensory Stimulation

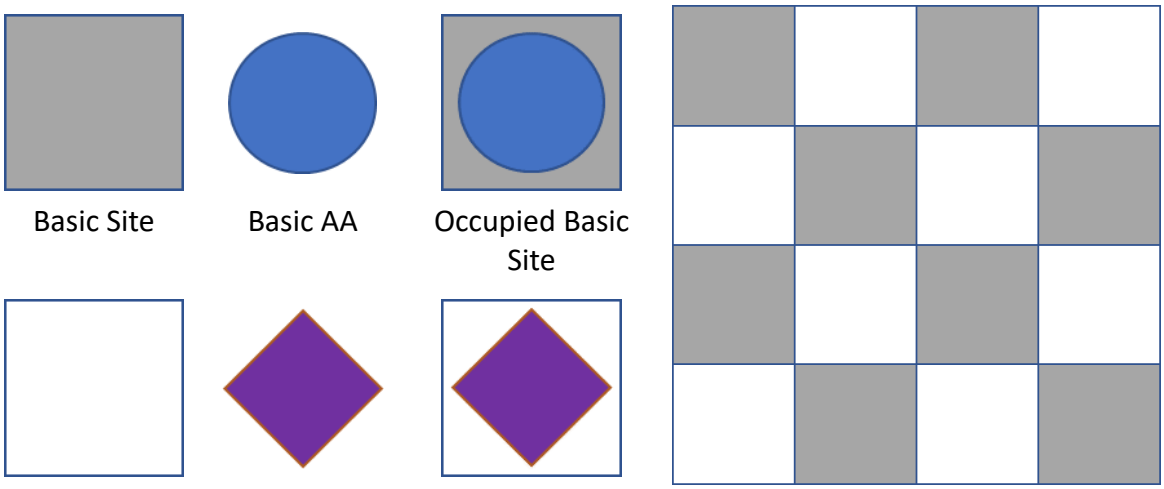
All living things possess chemosensory receptors – we are most familiar with those associated with taste and smell. These respective gustatory and olfactory receptors have evolved over millions of years to become extremely specific. For example, our vast array of individual specialized olfactory receptors can identify literally hundreds of thousands of individual odors – a clear evolutionary advantage literally every organism on the planet now enjoys. *Now enjoys* is the key phrase here. How did primitive organisms find food prior to the evolution of this sophisticated biochemical machinery? The answer is most likely through the detection of amino acids. As mentioned previously, all living things are continually excreting amino acids into their immediate environments, with certain fish species, as well as a host of simpler organisms, still

being able to utilize their amino acid receptors to follow trails of these AAs to their next meal. Significantly, in contrast to our modern olfactory systems, AA receptors only provide a simple 'yes' or 'no' signal for the presence of food, *not* information about its identity or condition – there would be no distinction between fresh strawberries and stale bread, for example, just an indication that something edible is present. As a result, for most modern organisms, their rudimentary AA receptors have been 'phased out' through later evolutionary processes. Importantly however, ancient fish species, that rely on an enhanced sense of smell to either find food (carp, catfish, sturgeon) or to navigate (salmon, eel), have retained their AA receptors, which continue to function as an integral part of these creatures' sensory armories - a fact we can now take advantage of…

Setting up the Board

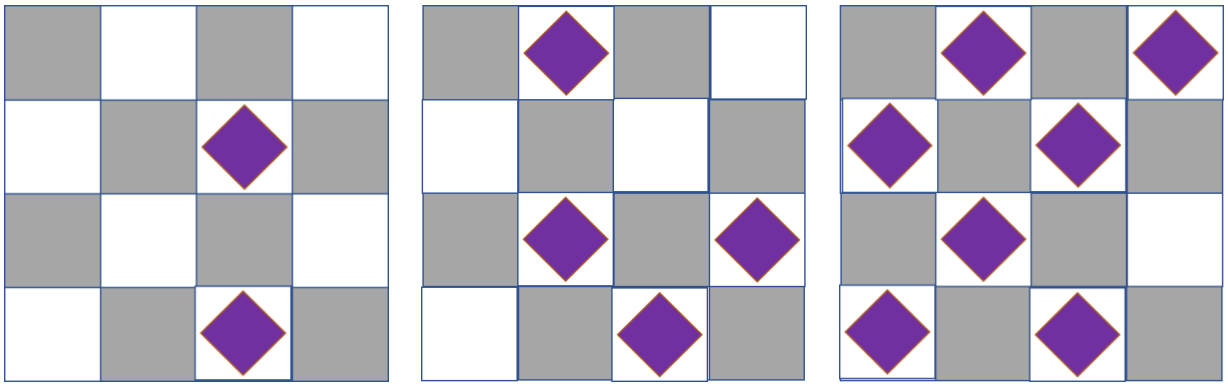
As stated above, by reading between the lines of *Carp Fever* it was clearly implied, and later confirmed by ours and others work, that carp are stimulated to feed by *either* Basic *or* Neutral amino acids but, crucially, *NOT* both. With these facts in mind, we can infer that carp possess two different types of AA receptors, dedicated to the respective recognition of Basic or Neutral amino acids, with these receptors essentially switching off when simultaneously exposed to both classes of AA. Without going into too much detail (see dedicated articles at the science sections of BioSourceBaits.com or AminoTec.com for full details), these observations are consistent with our novel Bivalent model (BVM) of chemosensory stimulation, which accurately describes the fishes' response to any single or mixture of amino acids, for any AA ratio, under any condition of concentration. This model has subsequently been used to optimize the most recent version of Impulse™, as well as lay the theoretical groundwork for upcoming species-specific attractants (Catatonic™, Cypriophil™) and hydrolysate additives (Hydrostim™). What follows is a summary of results furnished by the BVM with regard to describing *Carp Fever's* key observations and, as a consequence, how this information can be capitalized upon by anglers wishing to create significantly more attractive baits and feeds.

In order to fully appreciate and ultimately utilize the BVM, it is important to understand how this model emulates the physiological machinery associated with AA stimulation. Don't worry, this is much easier than it sounds, as a draughts board and its pieces make for a pretty decent analogy of what's actually going on 'under the hood'. First, as shown below, we are going to imagine the fishes' individual receptors as squares on our board, with the dark squares representing Basic AA receptors and the white squares Neutral AA receptors. As the fish encounter AAs, these sites will begin to fill with their associated AAs, generating a stimulatory response. In our model Basic AAs are represented by blue spheres, with Neutral AAs depicted as purple diamonds. We now have the tools we need, as shown by the following key:



Single AAs: Finding the 'Goldilocks Zone'

A key finding of pharmacology is that, for pretty much any drug-receptor interaction, a receptor site occupancy of ~50% is typically necessary for the onset of the desired therapeutic effect, with often unpleasant (overdose) side-effects seen for coverages above ~85%. This mirrors the observed 'zero-hero-zero' response we have observed, with single AA receptor site occupancy ranges, of 0% - 50%, 50% - 85% and 85% - 100%, as illustrated below, now attributable to the three respective phases of this effect. It is important to state at this point that the 'overdosing' of fish with amino acids is not in any way harmful to them - their feeding response simply 'turns off' in the presence of high concentrations of individual AAs.



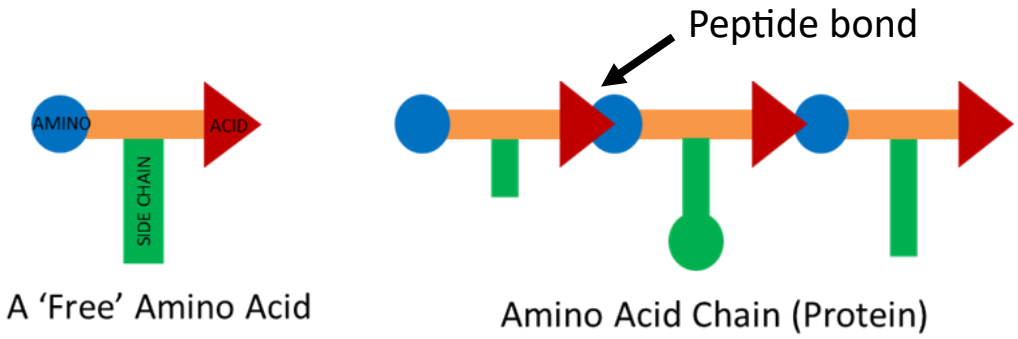
Importantly, being able to correlate the commencement of the 'just right' Goldilocks zone of AA receptor site coverage (~50%) with a specific threshold of single AA concentration (e.g. ~2.0 ppm for LYS), as just described, provides the essential theoretical foundation required for the creation of more effective dual amino acid stimulants, such as Impulse™. We'll pick this topic up in more detail later, but in order to do so we first need to familiarize ourselves with some more subtle, yet highly significant, molecular-level details of how amino acids bind at their respective receptors – a task that can also be accomplished through a deeper dive into *Carp Fever's* findings.

Carp Fever's results for single AAs can be further explained in terms of our checkerboard model. Since Maddocks' experimental technique, as previously mentioned, involved the introduction of a solution of AAs to a spot *under* the gravel in his test tank, their concentration, as well as their corresponding receptor site occupancies, consequently, began at zero before slowly transitioning, through perfusion into the tank, to stimulatory 'Goldilocks zone' (~50 - 85% coverage) levels over the course of each experiment. Importantly, it now seems clear that Kevin likely used relatively small amounts of AAs to make his test solutions, as only in doing so would the overdose condition be avoided. Significantly, only such a methodology would also allow for the creation of *Carp Fever's* signature league table of amino acid potencies. In more detail, whether or not an individual AA-receptor site interaction generates a stimulatory response is dependent on the amount of time the AA spends bound to the site, with a minimum residence time required to activate the receptor. A good analogy would be that of pressing the power button on your mobile phone – only a ~two second press will turn your device on – an important feature that prevents the device from being accidentally activated. Similarly, chemosensory stimulation will only occur if an AA exceeds a fixed minimum residence time at its receptor. Thus, if AA solutions of similar 'non-overdosing' concentrations are compared, as it is assumed Maddocks did, then Kevin's league table is, from a biochemical perspective, essentially listing the observed potencies of the AAs tested in terms of their associated residence times. While this 'nerdy' fact is of little practical use to anglers, an appreciation of the underlying molecular scale features of amino acids that determine any specific AA's residence time, and ultimately its potency, is of immense interest and relevance. This sounds like a lot, but it really isn't. As mentioned above, what appear to be complex biochemical processes are in fact, thank you nature(!), quite simplistic if viewed from the molecular perspective. So, let's break down what those 'sciencey' terms just introduced are *really* talking about, and, ultimately, how developing such a micro-level understanding of how AAs induce a feeding response can be transformative in terms of how we think about baits and their application…

Amino Acids 101

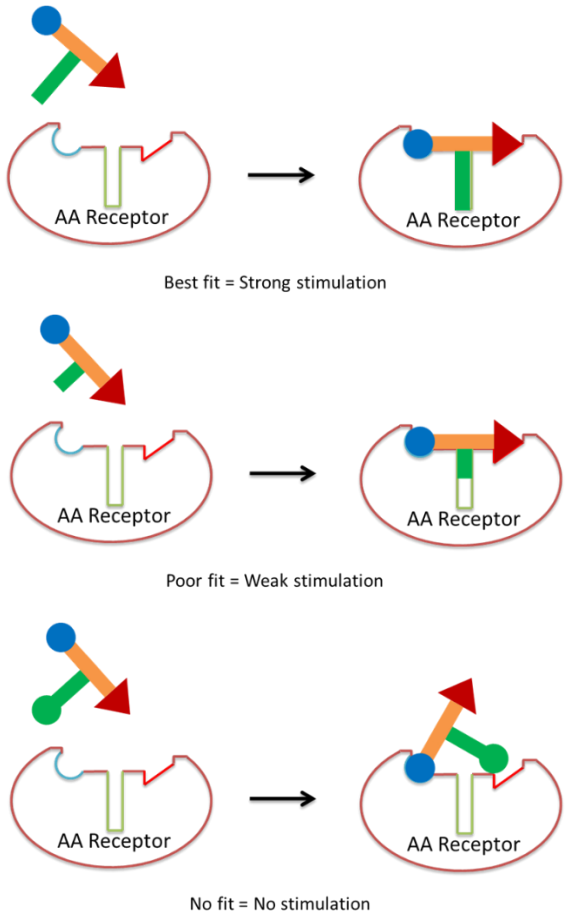
There are a lot of buzz words bandied about in carp circles, such as hydrolyzed protein, hydrolysate, 'free' amino acid, peptides, polypeptide etc., that add an air of scientific authority to a bait label. What do these terms actually mean and do they make a bait better? Let's jump in and take a look from the molecular perspective.

Amino acids are so named because they uniformly possess two common molecular features – a basic amino group and an acidic carboxylic acid group, which, as shown in the below diagram, sit on opposite ends of the amino acid's backbone. AAs only differ in terms of their characteristic *sidechains*, shown as various green shapes below, which, in turn, possess chemical groups associated with the Acidic, Basic, Neutral and Polar AA classes introduced previously. Crucially, each AA has a distinctive sidechain, with the size and chemical functionality of this sidechain essentially acting as a unique 'key' that will, ultimately, engage the respective AAs receptor site 'lock', so generating a stimulatory response – we'll return to this important concept in more detail momentarily.



As stated earlier, proteins are made up from long chains of amino acids, with each protein (see previous table) possessing a characteristic profile of all 20 AAs in slightly dissimilar relative amounts. In much the same way as the tops of Lego bricks can be repeatedly stuck to the bottoms of others to make a tower, the amino end of an AA can be attached to the acidic end of another through the formation of *peptide bonds*, ultimately, through repeated additions, creating longer protein chains. When digested and/or *hydrolyzed*, to create a *hydrolysate*, proteins are sequentially broken down into progressively smaller units. First, through *enzymatic* action, *polypeptides* and *peptides* are created - these shorter AA chains, respectively, contain between 100-700 and 2-50 amino acid subunits. A final *hydrolysis* step severs these species' remaining peptide bonds, forming the parent protein's characteristic profile of *'free' amino acids*. Hydrolysates may contain polypeptides, peptides, 'free' amino acids, or, most commonly, depending on the level of digestion, a combination of all three products. **Significantly, it is important to recall that only 'free' AAs are responsible for feeding stimulation, not the presence of peptides or polypeptides. Thus, fully digested 'hydros' have the potential to be more stimulatory than their partially digested counterparts, e.g. fermented or 'cultured' baits**.

As touched on above, the most important molecular feature of an amino acid, which ultimately governs its effectiveness as a feeding stimulant, is the nature of its sidechain, with stimulation being triggered through a 'lock and key' type interaction between the amino acid's sidechain 'key' and its dedicated receptor site 'lock'. Now, since we have established that carp are only sensitive to either Basic or Neutral AAs, it stands to reason that their chemosensory systems only feature dedicated chemoreceptor 'locks' capable of engaging AAs from these two specific classes. This not only explains *Carp Fever's* observation that acidic amino acids were consistently non-stimulatory, but also justifies the fundamental premise of our novel Bivalent (meaning 'to bind two') receptor site model.



Now, returning to 'the man behind the curtain' – the relationship between AA residence times and stimulatory potency. At the molecular level, residence times are essentially determined through a comparative measure of how 'sticky' a particular amino acid is towards its respective binding site – this is what biologists refer to as binding affinity, with greater affinities being correlated with higher residence times and more potent AAs. In simple terms, as also alluded to in our table of amino acid potencies, this can be simply rationalized in terms of how well the AAs sidechain 'key' fits its receptor site 'lock'. As further illustrated by the graphic, an excellent fit, as associated with, for example, Lysine at its basic site, results in a high residence time and a correspondingly significant stimulatory effect. Conversely, a poorer fit, as associated with, for example, proline at the basic site, will result in a diminished stimulatory response. An AA that does not bind to the site, because of an extremely poor fit, typically because it belongs to a dissimilar class, will not cause stimulation.

As previously mentioned, the potency of single AAs approximately span a factor of ten, with the 'poorest fit' AAs, such as Proline and Betaine, being ~10 times less potent than the most stimulatory 'best fitting' AAs, such as Lysine or Valine. With this important fact established, we can now add an essential detail to our previous assertion that "*the specific choice of (Basic or Neutral) stimulatory AA used is rendered largely irrelevant so long as… a very precise dosage is strictly employed*". Briefly, in practical terms, **'poor' AAs, such as Betaine, *must* be used at rates approaching 10x those of 'excellent' AAs, like Lysine, in order to generate a comparable stimulatory response**. This finding has significant repercussions in terms of the physical amount

of AA used by the angler, with ~2 mg of Betaine, rather than ~0.2 mg of Lysine, now required in and around the baited area to establish the necessary threshold AA concentration required for stimulation. Taking a typical mesh PVA bag of pellets as an example, in order to establish and *maintain* the necessary 2.0 mg Betaine in the baited area, the pellets, assuming a breakdown time of ~20 minutes, must be dosed with no less than an estimated ~40 mg - 60 mg of Betaine, where only ~4 mg - 6 mg of Lysine would be required. This may not sound like much in absolute terms, but in practicality it is significant. For example, literally 'just a couple of quick squirts' from an atomizer containing a Lysine solution onto our PVA bag would be sufficient to cause stimulation, while greater than 20 squirts would be required from a similar strength Betaine solution. **The take home message here for anglers is clear – while low potency AAs, such as betaine, *can* work well as fish feeding stimulants, the dosings required for common baits can often exceed practical limits**. Consequently, more efficient single AA stimulants, such as our original BioSource™ product, featured a primary high potency AA taken from the 'Excellent' class – this resulted in more manageable dosings, typically between 15mL and 30 mL of liquid per kilo of bait being needed to achieve an optimal feeding response.

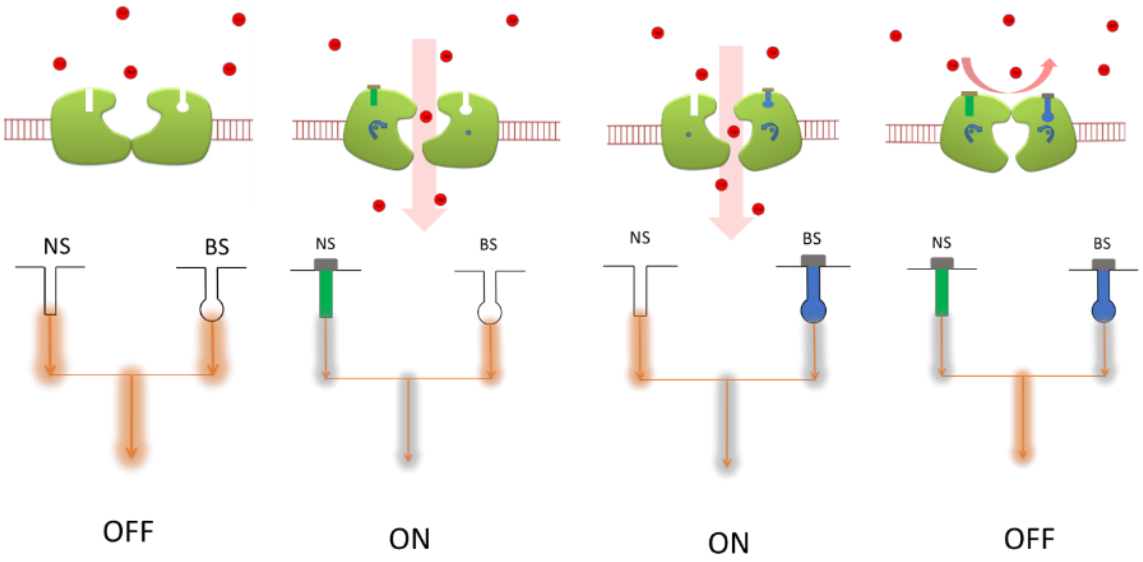
<u>In Search of the Grail</u>

The Holy Grail of fish feeding stimulants can be defined as a product that simultaneously satisfies two fundamental criteria: First, it would generate an optimal level of stimulatory response through quickly establishing the 'Goldilocks zone' (~50% – 85%) of receptor site coverage while, second, and most importantly, it would also be able to maintain this optimal level of stimulation through a broad dosing range. While single AAs can accomplish the first of these objectives, they have significant difficulties achieving the second due to the extremely narrow concentration (aka dosing) range associated with maintaining the optimal 'Goldilocks zone' coverage for single AA receptor sites. Being able to maintain the 'Goldilocks zone' over a broader dosing range was clearly the key to finding the Grail, but how? I wrestled with this dilemma for literally months without making much headway and, in all honesty, was about to throw in the towel until it struck me. If, as Maddocks had shown, that the most potent AAs from the Basic and Neutral classes essentially cancel one another's stimulatory effects when combined in similar amounts then maybe, just maybe, a *skewed* ratio of these two AA types would result in a *partial* cancellation of response with this effect, hopefully, emulating single AA type optimal stimulation over an extended concentration range?! I literally jumped out of bed, it was around 2:00 am as I recall, and scribbled down the beginnings of what would, ultimately, become our Bivalent Model (BVM) of chemosensory stimulation. Not quite Newton's apple, but this was certainly a personal eureka moment! Now, if you're good with the concept, those last few sentences are worth rereading as this simple realization not only underpinned the design of our first-generation dual amino acid feeding stimulants, but also, as is discussed fully below, provides answers to why fish are attracted to natural AA profiles, as well as why hydros should be used sparingly as bait glugs or soaks.

Henry's Diary

Our first experimental study testing the 'partial cancellation' hypothesis was completed by students at St. Olaf College (Minnesota, USA) back in 2011. This work clearly showed that the stimulatory properties of a Valine / Lysine mixture were indeed dependent on the ratio of these two AAs. Specifically, it was demonstrated that a near equivalent Valine to Lysine ratio resulted in a negligible stimulatory response, while blends possessing greater relative fractions of either amino acid became progressively more stimulatory, ultimately returning a better response than our original single AA product (BioSource™). These results not only validated, but also allowed for the quantification of *Carp Fever*'s observation that: "amino acids which were best on their own, proved to be ineffective when used in combination". Crucially, based on these findings, we were able to put the final touches to our Bivalent Model (BVM) - we now had access to a fundamental understanding of the actual molecular scale events leading to AA induced stimulation, an essential first step in realizing the ultimate goal of developing the 'Holy Grail' of fish feeding stimulants. For those familiar with the Indiana Jones movies, you'll recall that the *Last Crusade* detailed how Drs. Jones ultimately used Henry's diary as a guide to find the Holy Grail. In terms of our discussion, the BVM can in many ways be considered analogous to Henry's diary, as its application has ultimately led to the development of our range of next generation 'Holy Grail' feeding stimulants.

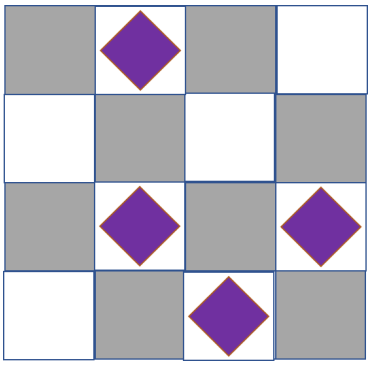
Now, what follows is an important but somewhat complex 'sciencey' discussion of how the BVM models chemosensory response, so readers who are mostly here looking for bait hints and tips, and not so much for an impromptu biochemistry lesson, may wish to skip to the next section. OK, let's take a deeper dive and explain exactly how we think the BVM emulates the stimulatory properties of AA mixtures. First, the Bivalent model, as its name suggests, relies upon the fundamental assertion that each of the fishes' AA receptors possess a pair of Basic and Neutral binding sites. This is a significant departure from the standard model of chemoreception, as illustrated in the 'Amino Acids 101' section, where receptor sites are generally considered to be monovalent, or only capable of binding a single, specific type of molecule. This makes absolute sense in terms of our highly evolved olfactory systems, as when we encounter fresh strawberries that's what we need to smell – not the odor associated with stale bread! As also detailed in the previous 'Popping the Hood' section, the AA receptors' original task was, most likely, to provide primitive organisms with a basic 'yes' or 'no' signal for the presence of food. Crucially, this goal can only be achieved, without overdosing, if the fishes' receptors are, in fact, bivalent. As shown by the following graphic (apologies in advance, as this figure was taken from a scientific article and assumes a certain level of specialized knowledge), only such a model returns the required 'on' and 'off', conditions, respectively, as per *Carp Fever*, for single AAs and mixtures of AAs. In more detail, the BVM accomplishes this task by essentially treating the Basic (BS) and Neutral (NS) receptor sites as inputs into, what electrical engineers refer to as an EOR ('exclusive or') Boolean logic gate. Given that nerve impulses are essentially generated through the creation of biochemically derived voltage differences across a cell's membrane, such an electrical circuit type model then seems to be both reasonable and appropriate.



Bivalent chemoreceptor (top) and equivilent EOR logic response for 'single' and 'double' AA binding events

<u>The Rules of the Game</u>

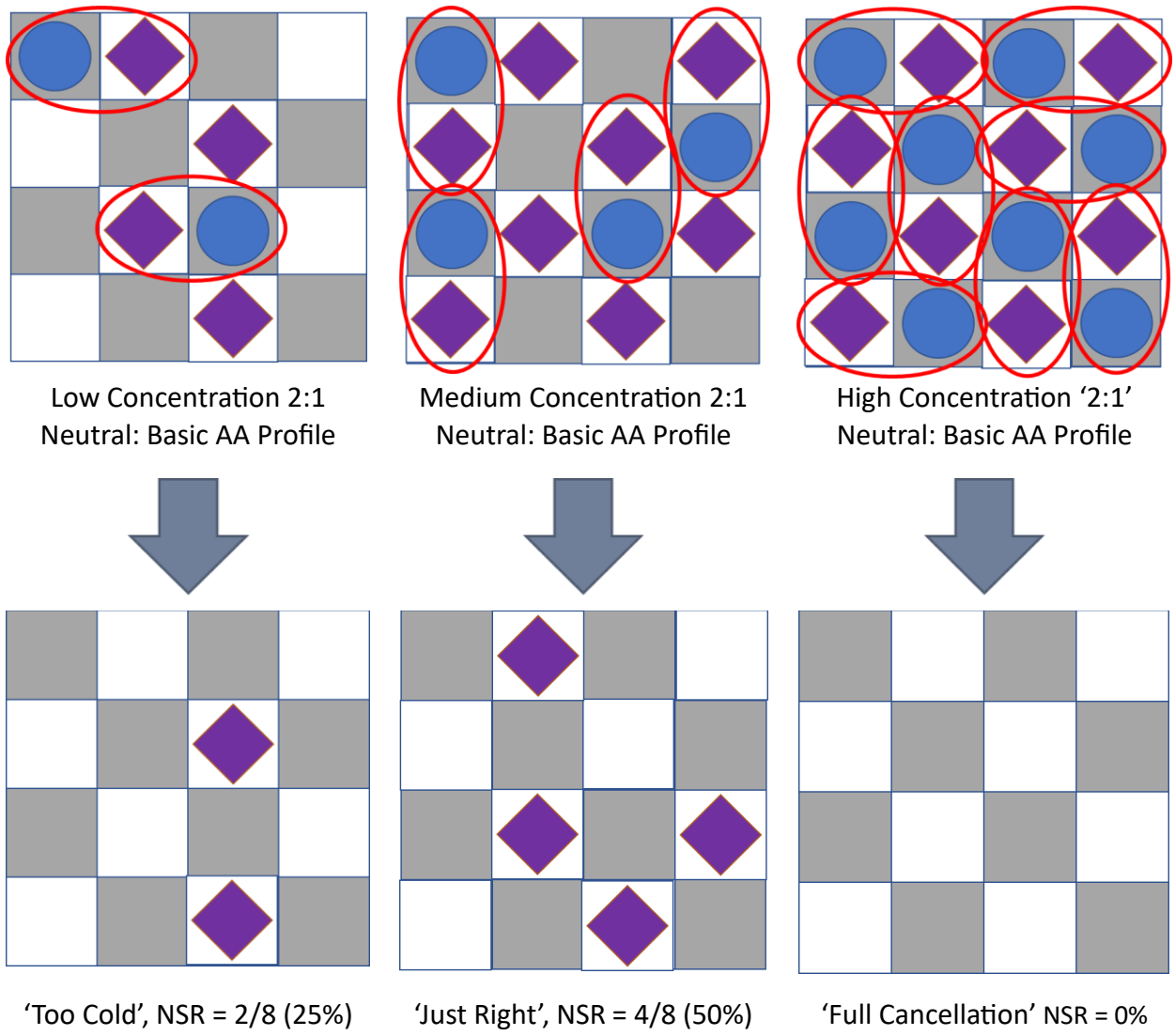
As detailed above, and welcome back if you skipped ahead, the key takeaways from the BVM are, simply, that when either *only* the fishes' Basic or Neutral sites are occupied then stimulation will occur (single AAs are stimulatory), while, when *both* sites become equally occupied, stimulation is terminated (AA mixtures are non-stimulatory). These modelled responses, while also clearly emulating Maddocks' and others experimental results, essentially provide us with the underlying 'rules of the game' relating to AA induced stimulation.



Returning to our simple checkerboard model, we can now better visualize how these rules allow for the determination of what we call Net Stimulatory Response (NSR) for AA mixtures. A quick recap: Basic and Neutral binding site 'locks' are represented by dark and light squares, with their corresponding Basic and Neutral AA 'keys' represented by blue circles and purple diamonds. Our model board contains eight of each type of binding site. As shown again here, a single AA coverage of ~50% (4/8) is correlated with the stimulatory lower limit of the 'Goldilocks zone'.

Now, as is detailed below for a 2:1 blend of Neutral to Basic AAs, since equal amounts of Basic and Neutral AAs effectively cancel one another, as shown by the red lassos, they can be removed *as pairs* from our board. Consequently, the remaining non-cancelled excess of the dominant AA, which essentially now mimics an equivalent single AA coverage (as shown above), is responsible for the generation of a corresponding net stimulatory response. **In many ways,**

that rather lengthy last sentence encapsulates the key finding of close to half a lifetime's worth of research – *it is the partial cancelation of response, through the generation of AA pairs from dissimilar classes, within any AA blend or profile that ultimately renders such mixtures stimulatory*. As shown below by the following graphic, the 'equivalent single AA response' (NSR) for literally any AA blend of known Neutral to Basic AA ratio, for any coverage (i.e. equivalent dosage or concentration) can now be determined:



The above graphic nicely illustrates how and why fish respond to AA profiles derived from natural food sources, plus also provides a warning as to why hydros should be used sparingly as glugs or soaks. First, as shown by the left column, the resultant small NSR associated with a low concentration AA blend, i.e. the fishes' 'first whiff' of food from a great distance, is correlated with the commencement of searching behavior. During this phase, as also witnessed in lab, fish will orient themselves to, and swim into, the trail of AAs emanating from a food source. As the

concentration of the food's AAs increases, the NSR will also correspondingly increase, entering the 'Goldilocks zone', as shown by the second column, ultimately initiating a full on 'tails up' feeding response. At this point, the fish will have located the food source. Finally, we see how clever nature really is (and why using hydros might be a bad idea). When a fish is basically 'on top' of the AA source (i.e. food), it's receptors will become filled to capacity, or saturated, as shown in column three. Now, if these were a single class of AA the fish would become overdosed, with their feeding behavior shutting down. However, for any AA blend, as also illustrated by the final column, overdosing is avoided through the fish effectively turning off their AA receptors. This makes absolute sense, as if the fish has located, and is actively 'troughing' its food, then the receptors' job is complete, with any further distraction from feeding not being evolutionary advantageous. Importantly, because hydrolysates (e.g. CSL), plus commercial AA blends, such as Minamino, typically contain super high amounts of free AAs, they will likely transition the fish to the 'full cancellation' condition at some distance from the anglers' bait. **Thus, for anglers wishing to invoke a feeding response around their bait, glugs or soaks should feature minimal amounts of hydrolysates, or should exclusively feature a dedicated concentration independent feeding trigger, such as Impulse™**. Having established these facts, it's important to state that it is theoretically possible to render hydrolysates highly stimulatory through the addition of proprietary 'site-blockers'. As is discussed in the final 'Putting it all Together' chapter, the precise application of 'site blockers' to any single amino acid (Impulse™, Catatonic™) or multi (Hydrostim™) AA blend will render it optimally stimulatory.

<u>It's Ladies Night!</u>

If you found the information contained within that last section as eye opening as I originally did, then, in the words of Obi-Wan Kenobi, 'that's good, you've taken your first step into a larger world' – congratulations Padawan! So, if you feel comfortable with the concepts just presented, then feel free to skip to the next section. If, however, if that was all just a little too 'sciencey', don't worry, I have a great marginally dodgy nightclub analogy that may make more sense…

So, you own a nightclub that has a maximum occupancy of 16 people (total number of receptor sites). Now, by the end of the night you want to have the club at full capacity with equal numbers of guys and girls having a good time (saturation coverage, all 8 neutral and 8 basic sites occupied). However, you want to make a little cash before the pubs kick out, so you put on 'Ladies Night'. As a result, you get a few single ladies (🔷), as well as a few couples (🔷🔵) come in earlier (low concentration condition). Your secret plan is working, you now have a small excess of female guests, but it's not quite enough of a difference to get more guys in ('Too Cold'). The night progresses, and just before last call you've attracted just the right mix of couples and single women ('Just right'), prompting the guys to make their way over. The night ends up just as you had hoped (high concentration), with equal numbers of guys and girls 'getting to know each other', OK, hooking up (🔷🔵). Everyone is happy (full cancellation), as you kick back, count the night's take and start dreaming about that beach in Marbella….

Part 3: Putting it all Together

Want to Buy Some Golden Eggs?

In 2012 the hard work of putting it all together, with the ultimate objective of creating a practical fish feeding stimulant capable of meeting our two prerequisite 'Holy Grail' criteria, really began. What became our first successful dual AA product, code name 'Jigsaw' (later rebranded Aminoplex™) utilized a proprietary blend of Basic and Neutral AAs to successfully achieve optimal levels of stimulation over a broadened dosing range. Now, having a goose that lays golden eggs is one thing, convincing people these really are golden eggs is something else entirely. 'What's he on about now?' you are rightly thinking. Well, that last sentence about the Golden Goose really sums up my experiences trying to convince bait companies that we really did have this *magical* powder that doubles catch rates. In hindsight, I totally understand the skepticism, as I'm sure bait companies are approached 'on the regular' by anglers trying to peddle their own home-grown concoctions. However, one company, Richworth, did take a chance on us. Working with the awesome Pete Wilson, we developed a porous base mix incorporating, what would later become, the Aminoplex™ additive. Pete sent the resulting prototype boilies, as well as some undosed 'control' versions, out to his most trusted testers. While not a surprise to us, everyone on the Richworth side were completely blown away by the results, with a better than 2:1 catch rate recorded for the Aminoplex™ baits over the otherwise identical undosed versions. Richworth were now onboard, with Pete and I now tasked with developing a full range of what would later become the S-Core product line.

With the S-Core line now in production, and slated for release in the spring of 2013, I convinced Pete to supply my contact at Sparsholt with some of our prototype boilies for testing. Student Ollie Ricotti's resulting dissertation "An investigation into the effectiveness of 'Aminoplex' bio-stimulant as a feeding stimulant in a controlled angling environment" confirmed Richworth's findings, concluding that: "The 'Aminoplex' treated baits accounted for 69% of the 'bites', which can be expressed in the ratio 2.2:1. There was a strong relationship present between the bait used (dosed or control) and the bite numbers experienced". In common with the Richworth tests, Ollie's trials also featured anglers utilizing a two-rod approach, with dosed and undosed baits each fished on their own rod. The actual results of Ollie's dissertation are reproduced here. Additionally, as mentioned previously, a copy of the original thesis can be viewed or downloaded at either the AminoTec.com or BioSourcebaits.com websites. Interestingly, as illustrated by the accompanying photo, Aminoplex™ was able to coax fish into feeding under the harshest of winter conditions. This illustrates a wonderful side-effect of the products, which we have since witnessed consistently - they have the ability to switch fish on to feeding at very low temperatures, which is great news for anglers who like to fish year-round.

Trial	Number bites from **Aminoplex**	Number bites from **Control**
1	10	4
2	6	4
3	3	0
4	7	8
5	12	3
6	13	6
7	4	3
8	11	4
9	6	2
10	9	4
Total	**82 (69%)**	**38 (31%)**

The Fall of Rome and The Dark Ages

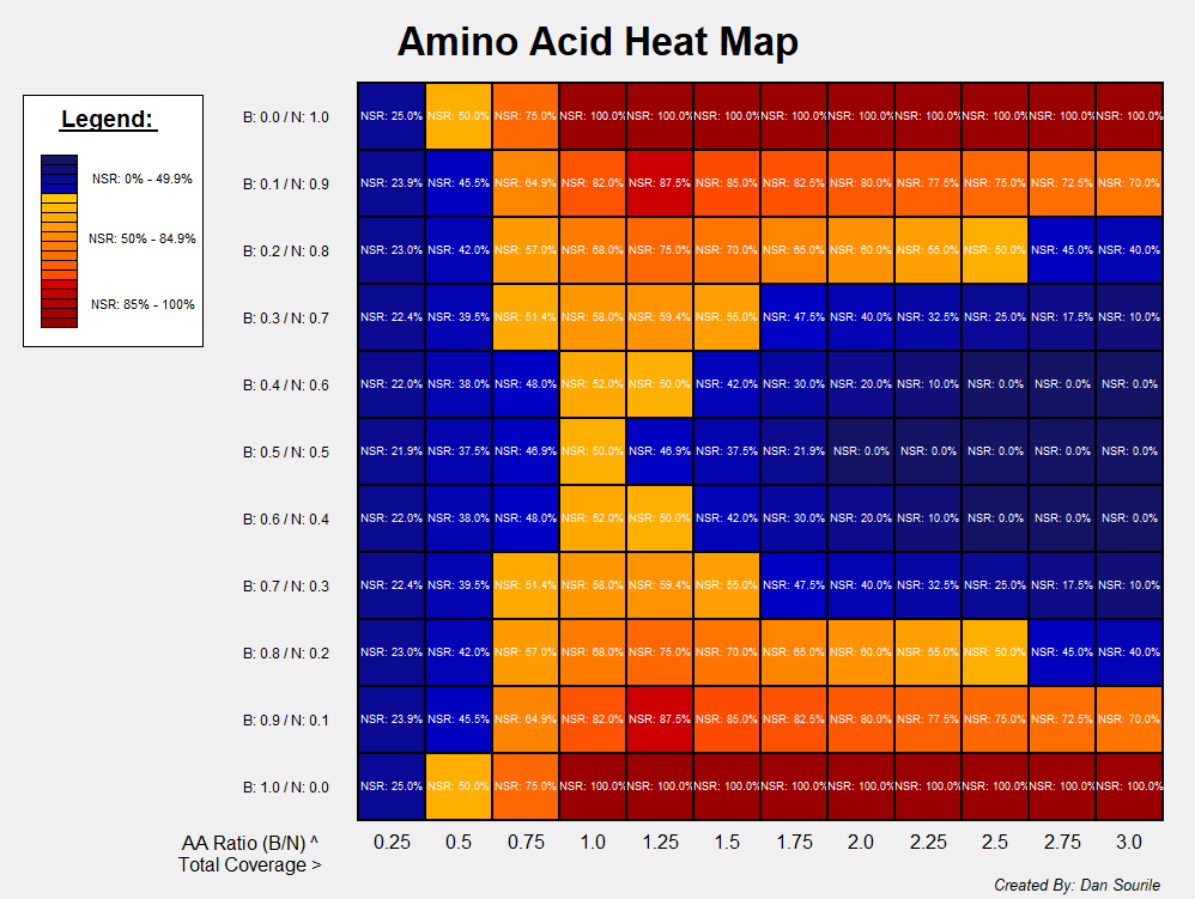
After its launch in 2013, the original S-Core range really started to make a splash – you may recall Martin Bowler's *Seeking Shadows* books and DVDs, with the DVDs in particular, as narrated by the late Bernard Cribbins, extolling the virtues of a new scientific 'wonder bait'. Unfortunately, it is no longer possible to buy any of these original Aminoplex™ dosed products. Briefly, soon after Bob Baker, the legendary Richworth founder, entered retirement the company went into something of a slow decline, eventually being sold to a third party. While it is still possible to find S-Core branded products online and through select retail outlets, ***these products no longer contain the Aminoplex™ additive***, and, as a result, have basically become just another run of the mill boilie. At the time, this felt like the Fall of Rome, as all that we had built now seemed to be coming down around us. In common with what is becoming an admittedly somewhat overly dramatic historical analogy, with our main business partnership now gone, we too then suffered through our own Dark Age while trying to figure out what to do next. Fortunately, as discussed in the next section, this down time afforded us the breathing room we needed to revisit the fundamentals of the Bivalent Model and, ultimately, experience our own Renaissance with the creation of a totally new class of ground-breaking products.

The Renaissance

While simple dual amino acid stimulants, such as Aminoplex™, do indeed create the desired optimal level of stimulation over an extended dosing range, this range does have limitations. Importantly, we have since been able to determine, using a more rigorous statistical version of the Bivalent model, the practical boundaries of this 'Goldilocks zone'. Now, this next part is about as detailed as the science gets and may, or may not, be of great interest to the casual reader. However, for those wishing to *really* understand how chemosensory stimulation works what follows will certainly provide those answers. Having said that, it is possible to get a good

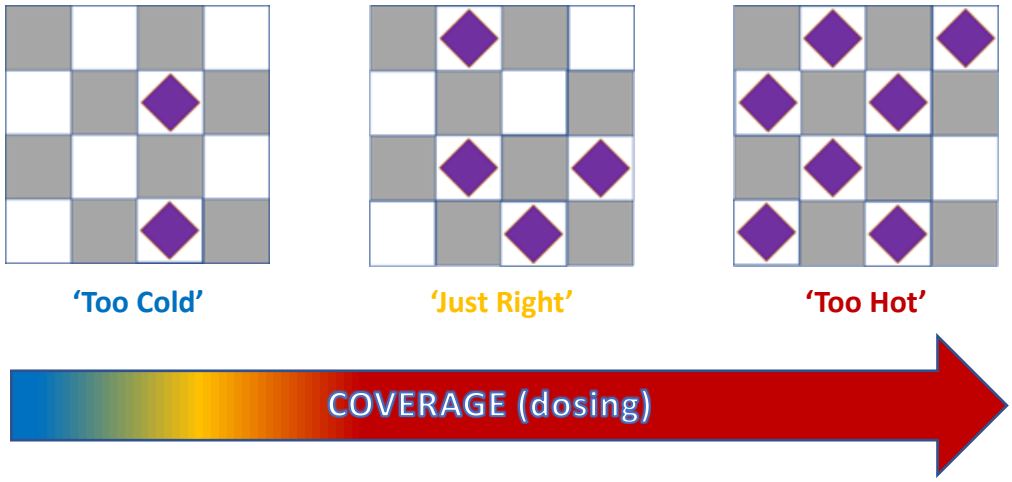
grip on the fundamentals through reference to our 4x4 checkerboard model, which we will continue to reference, as the full statistical model is essentially based on a similar, but larger, 8x8 bivalent receptor grid. In more detail, Net Stimulatory Response (NSR) is determined through calculating the statistical distribution of 'empty' ( ), 'half full' (or) and 'completely full' ( ) receptors, with the fraction of 'half full' or singularly occupied sites (recall the EOR gate interpretation above) ultimately determining the corresponding NSR. However, since, say, a Neutral AA has a 'choice' as to whether it binds to either an 'empty' or a 'half full' receptor, the corresponding distribution of respective 'on' and 'off' signals from these individual receptors will generate a 'smoothed out' or statistically broadened net response.

If the Renaissance's greatest work is assumed to be the Mona Lisa, then ours is undoubtedly the Heat Map. As shown below, the Heat Map, created in collaboration with mathematician Dan Sourile, provides the NSR of any AA blend as a function of Basic: Neutral AA ratio under any condition of dosage or coverage. **The ramifications of this finding are significant, as AA blends possessing optimal stimulatory properties can now simply be 'looked up', so negating the massive effort associated with completing literally hundreds of individual experiments or field trials**.

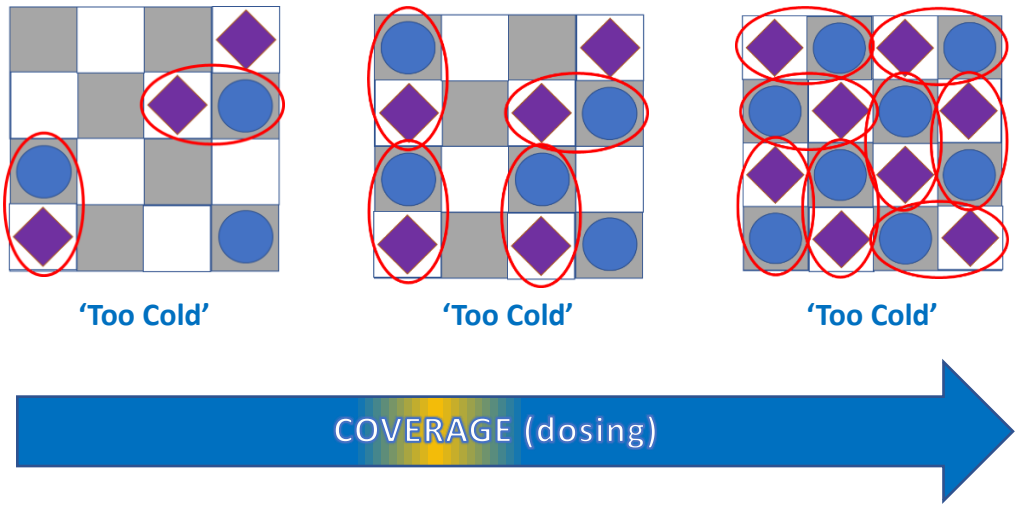


In more detail, the ratio of Basic: Neutral AAs is plotted on the Heat Map's vertical scale, with the coverage or dose of any particular blend running across the horizontal axis. For each permutation of AA ratio and coverage, respective blue and red squares indicate conditions of either a 'Too Cold' (NSR < 50%) or a 'Too Hot' (NSR > 85%) response, with the 'Just Right' 'Goldilocks zone' of stimulation (NSR = 50% - 85%) represented by the yellow and orange shaded areas. Incredibly, the 'Heat Map' validates essentially all ours and others previous experimental findings and predictions. Specific, key highlights include:

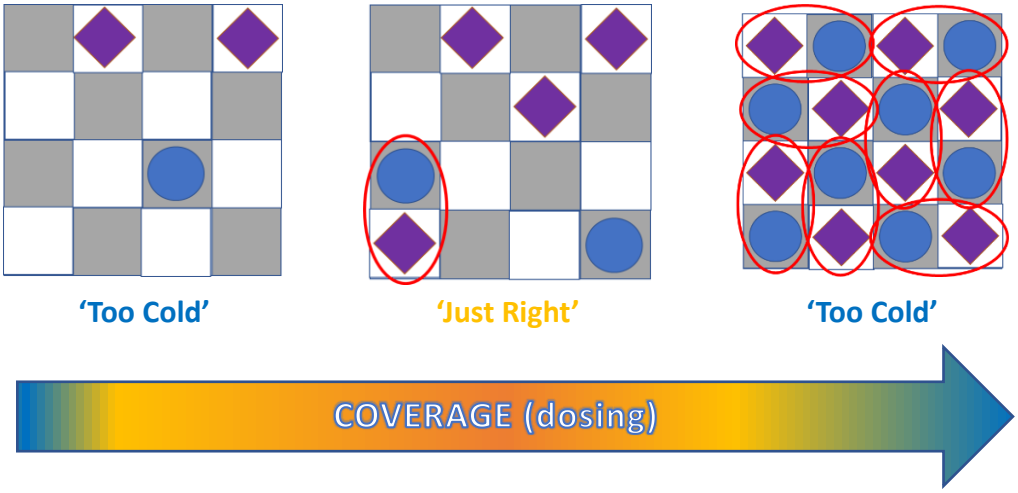
1. Single AAs: The rows relating to either the individual Neutral or Basic AA classes, top (B:0.0/N:1.0) and bottom (B:1.0/N:0.0), respectively, confirm that 'singles' have an extremely narrow window of optimal stimulation (NSR). This validates our reasoning put forward in both the above 'Zero-Hero–Zero' and 'Back to the Drawing Board' sections, that provided details relating to the practical limitations of our first single AA embodiment (BioSource™), as well as supporting Ken Townley's observations regarding the use of Betaine. Combining our original checkerboard model with the pertinent rows from the Heat Map, as summarized below, now furnishes a complete understanding of the stimulatory properties of single AAs:



2. The Equivalent Ratio: For an exactly equivalent (B:0.5/N:0.5) blend, the Heat Map's corresponding central row confirms our own, as well as *Carp Fever's*, experimental findings that such blends are essentially non-stimulatory over their entire dosage range. As is demonstrated by the below graphic, even though the fraction of each AA type within the blend is identical, they will generate a small proportion of non-adjacent 'half full' or 'on' receptors ( or ) in addition to the expected majority of 'completely full' or 'off' occupancies (). Thus, while the NSR essentially never reaches a stimulatory level, it will only truly fall to zero at higher coverages.



3. Optimal Ratios: For highly skewed AA ratios, such as the (B:0.2/N:0.8) blend shown by the Heat Map's third row, the 'Goldilocks zone' of optimal stimulation becomes noticeably extended through the entire low dosage range. This significant broadening, as illustrated by the below diagram, is rationalized through the fact that such slanted AA ratios generate a higher proportion of stimulatory 'singles' ( ) in concert with an ideal lesser fraction of cancelling 'doubles' ( ):



As you most likely have already guessed, our first true dual amino acid stimulant, Aminoplex™, was later confirmed to contain an essentially identical optimized AA ratio to that predicted by the Heat Map. This is significant, as the original Aminoplex™ recipe was originally arrived at through literally months of field trials and associated tinkering. Thus, the fact that the experimentally determined and calculated optimized AA ratios were later found to match provides both strong and irrefutable evidence supporting the validity of the Bivalent model.

Thus, without wishing to sound immodest, we can now be confident in our assertion that the amino acid code has finally been 'cracked', with the Heat Map ultimately illuminating the path to the further development of, as is discussed in the following section, a greatly enhanced, more effective range of species-specific fish attractants and feeding stimulants.

Giving Away the Farm?

You might be sitting there thinking that I've just 'given away the farm' by telling you exactly how to create your own commercial grade amino acid feeding stimulant. Well, yes and no. Briefly, attempting to establish an *effective* 8:2 ratio of aminos by simply mixing 8 and 2 grams of a couple of AAs together simply doesn't work, as a host of other 'nerdy' variables including AA type and potency, molar mass, molar solubility and pH must also be factored into determining an equivalent mass ratio. More importantly, and this is largely why I now feel comfortable sharing so many details regarding the development of Aminoplex™, this work has since been eclipsed through the development of a far superior class of stimulants featuring a new type of proprietary 'site blocking' species. These next generation feeding stimulants, as is discussed in the remainder of this article, are essentially able to lock in the 'Goldilocks' zone' of optimal stimulation through the entire dosing range and, in doing so, overcome the higher dosage limitations associated with previous dual AA embodiments, such as Aminoplex™.

Part 4: A Brave New World

More is *Not* Better

One interesting trait of human nature is the concept of 'more is better'. This usually holds up in our everyday lives – two beers down the pub are better than one, a couple more hours of overtime results in a bigger paycheck etc. However, if you take a quick glance back at the Heat Map, you'll see that an increase in the dosage of amino acids, either as singles or mixtures, ultimately results in a lessening of optimal response, or, in other words more is *not* better as far as aminos are concerned. This fact, more than any other, reinforces the concepts first discussed in the 'Zero-Hero-Zero' section, where it was revealed that the 'Goldilocks zone' of optimal stimulation can only be achieved through the maintenance of precise, miniscule amounts of AAs throughout the baited area. This actual dosage was calculated to be ~0.2 mg for Lysine, or in other words ~1/400th of a teaspoon! Clearly, this amount falls far below the threshold of measurement for most anglers, with a more 'common sense' amount, such as half a teaspoon of AA powder(s), clearly pushing way past the 'Goldilocks zone'. The 'more is better' mindset has also proven to be something of an Achillies heel to our previous BioSource™ and Aminoplex™ embodiments as, even though these products worked exceptionally well when used at recommended levels, anglers were often tempted to ramp up dosages in an attempt to

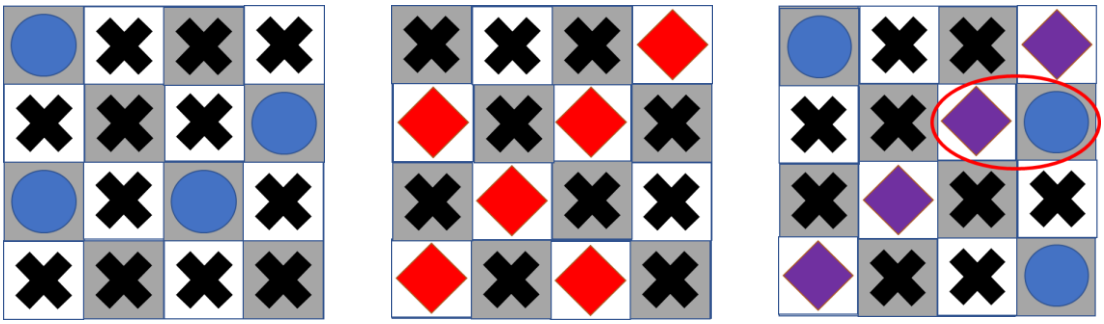
catch more fish, with the reality of this situation being that they'd inevitably experience noticeably diminished catch rates. From a philosophical perspective, battling human nature is clearly unwise, since, as just detailed, the resulting outcomes are typically counterproductive. Thus, in an attempt to create a more user-friendly product, we once again returned to the drawing board in search of what seemed like the impossible - a feeding stimulant whose stimulatory properties would remain unchanged through the entire dosage range. This ultimate embodiment seemed like a huge ask, but after another string of restless nights, now sprinkled in with a few 'are you alright?' queries from an extremely understanding wife, a breakthrough was, eventually, forthcoming. The answer, as it turned out, in common with so many notable discoveries, was deceptively simple. Briefly**, through the addition of a competitive 'site blocker' to any AA, the resulting blend's stimulatory properties will now *only* become dependent on the ratio of these two species, *not* the mixture's concentration. This finding, fully detailed below, is 'massive', as in one fell swoop, we have been able to dispense with the restriction that anglers are required to follow strict dosing protocols when using AA based feeding stimulants**. In other words, more *can* now be better as far as our next generation products, such as Impulse™, are concerned…

The Next Generation

While the many restless nights mentioned above ultimately yielded a potentially game changing piece of theory, this would have been all for nothing without being able to first identify, and subsequently confirm, the prerequisite chemical properties of an effective site blocking species. Without going into too much detail, I really had to reach back into my chemist's bag of tricks for this one, eventually settling on several worthy candidates. Multiple rounds of field trials and tank tests later both proved the concept and allowed for the identification of what we now simply call our 'Proprietary Blocking Agent' (PBA). Now, I'm unfortunately going to have to keep my lips sealed regarding the PBA's identity, as, in commercial terms, it provides us with a distinct competitive advantage. However, don't despair, as a full range of 'next generation' Impulse™ and other branded products containing this revolutionary technology are available for purchase via Biosourcebaits.com, as well as through a variety of other retail partners.

Well, 'that all sounds good' you may be thinking, 'but how does it even work'? Here's a great analogy. Imagine you have a truckload of draughts (checkers) – half of them are black and half are white. You dump what are literally thousands of these respective pieces over a 64 square checkerboard. Now, you tell me, on average how many squares will have a white piece on them and how many a black one? If you said half (32) each you'd be right. The massive number of draughts poured onto the board is irrelevant, as only their ratio determines the final outcome. In terms of a feeding stimulant, we previously estimated that the 'Goldilocks zone' corresponded to an equivalent single site coverage of between 50% - 85%. Thus, by simply creating an AA/PBA blend that generates this ratio, an optimal level of stimulation should be

maintained through the entire dosage range. This concept is nicely illustrated by the following modified 4x4 checkerboard models, with the PBAs simply represented by black crosses (✖):



Impulse™
Basic AA / PBA Blend
(NSR = 50%)

Catatonic™
Acidic AA / PBA Blend
(NSR = 75%)

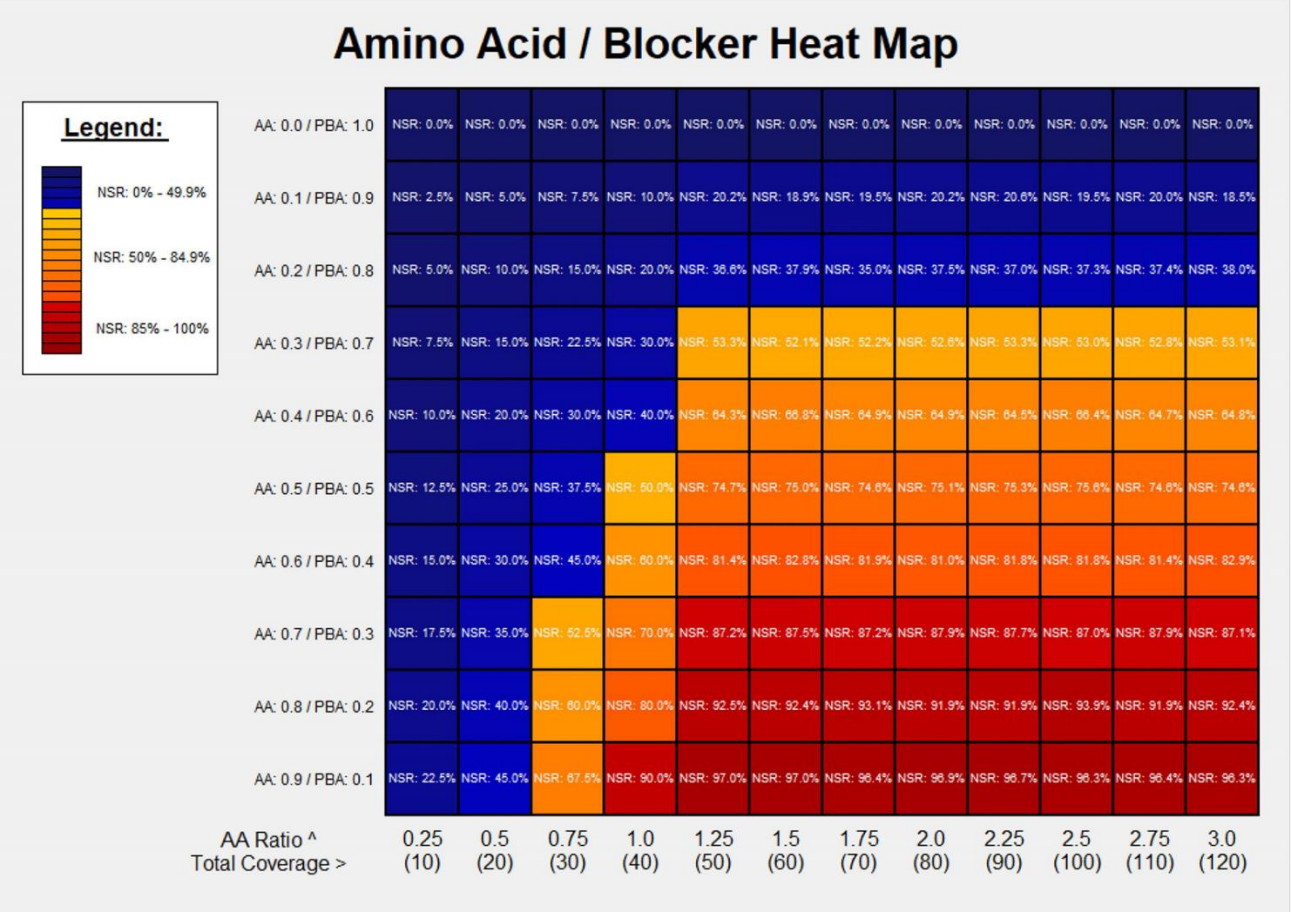
Hydrostim™
Mixed AA/ PBA Blend
(NSR = 63%)

A couple of quick asides, associated with the use of PBAs, before we move on. First, PBAs are assumed to not activate either the fishes' Neutral or Basic receptor sites, they simply 'cap them off' (an antagonist in biochemical terms), so no dual occupancy cancelling, as is seen for AA blends, such as Aminoplex™, is to be expected. Second, since it is assumed that the PBAs block both the Basic and Neutral sites, then any optimized single AA / PBA blend will unavoidably be slanted towards an excess of PBAs, as these species are programed to 'cap' both types of AA binding site. OK, back to it…

Significantly, as also illustrated by the above graphic**, the use of PBAs opens the door to a variety of exciting new species-specific and hydrolysate-based embodiments**. In more detail, *Carp Fever's* amino acid league table, as well as a large fraction of the materials so far discussed here, confirms that carp are primarily stimulated to feed by AAs from either the Basic or Neutral classes, with aminos possessing either Acidic or Polar groups being less or non-stimulatory. Thus, carp focused blends (e.g. Impulse™), will typically feature PBAs mixed with either Basic or Neutral AAs. Importantly, however, it appears as though catfish are very likely sensitive to amino acids from all four classes, meaning that a catfish-specific (Catatonic™) attractant can be constructed through combining either Acidic or Polar AAs with a PBA. Finally, and **this will likely be of great interest to anglers who routinely use 'hydro' based glugs and soaks, it is speculated that through adding just the right fraction of PBAs to any hydrolysate, the resulting mixture (Hydrostim™), as shown by the final panel above, will be rendered highly stimulatory. While our hydrolysate work is, at the time of writing, still in a preliminary phase, initial results do look promising**.

The Sistine Chapel

You may have been mumbling to yourself earlier, after reading that I'd assumed the Mona Lisa to be the Renaissance's greatest work: "hasn't he heard of the Sistine Chapel?" Well, I tend to agree with you, as, in terms of our analogy, I had mathematician Dan Sourile 'grab his brush and head up the ladder' to get started on his next masterpiece - an updated Heat Map dedicated to diagraming the stimulatory response of any single AA / PBA blend. Dan, once again, did not disappoint, with the resulting Amino Acid / Blocker Heat Map, shown below, clearly illustrating that, beyond a low threshold coverage, the 'Goldilocks zone' is maintained indefinitely through higher dosages for specifically defined AA: PBA ratios. For example, our new Heat Map predicts that an AA: PBA ratio of ~3:7 should generate an optimal Net Stimulatory Response of ~53% across the entire upper dosage range. Importantly, in common with our original dual AA Heat Map, the theoretical data provided by the below AA/Blocker version also closely emulates its corresponding experimental results, so further validating the authenticity of the Bivalent model.



Breaking the Dosage Barrier

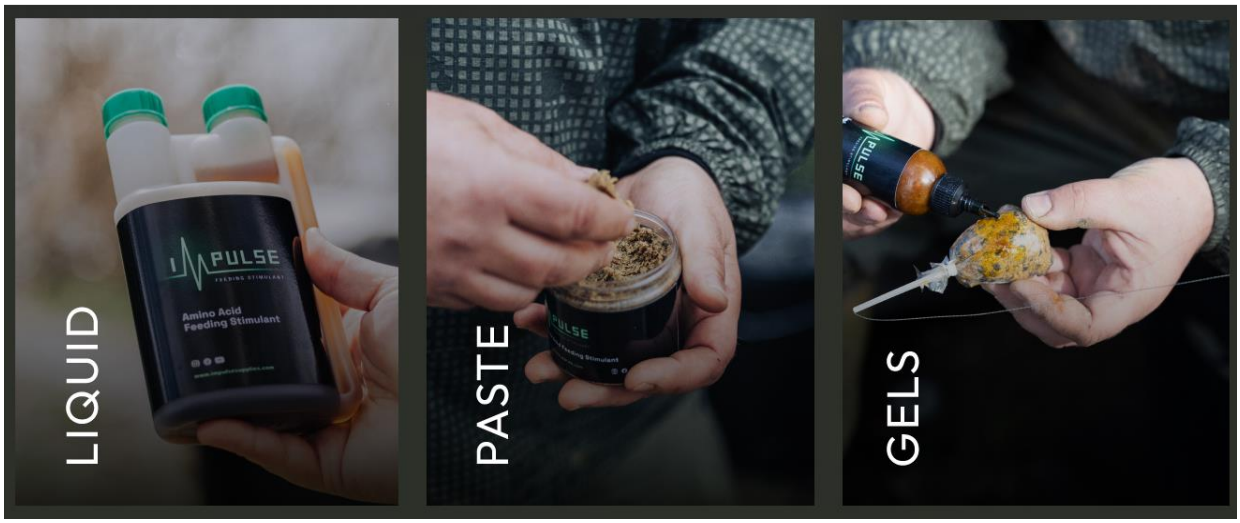
The most significant finding of this work is, of course, that, for specific AA: PBA ratios, the 'Goldilocks zone' has now become essentially limitless. My research students and I became somewhat dumbfounded when we first witnessed this effect as, to our astonishment, we were seeing a constant optimal response over a concentration range in excess of 1000 times that we had previously established for our first-generation dual AA blends. Given that our previous single and dual AA embodiments were only optimally effective through a narrow, strictly defined milligram range, and that we were now witnessing solutions with AA loadings approaching ~100 grams or greater returning a consistent optimal response, this really was 'a brave new world', as we had essentially just broken the 'dosage barrier'. **The development of such dosage blind embodiments, whose stimulatory properties remain optimal though the entire high dosage range, as stated above, is indeed 'massive', as users are no longer shackled by the necessity to follow strict dosing protocols when preparing their baits and/or feeds**.

With the dosage barrier now broken or, in terms of our continuing analogy, with the Sistine Chapel's ceiling now freshly painted, it finally became time to really show the world what we'd accomplished and where we were heading: the design, development and implementation of a range of Impulse™ branded fish feeding stimulants that really 'do what they say on the tin'…

Mission Accomplished?

While the work detailed throughout this report certainly provides a roadmap to the development of a range of commercially viable fish feeding stimulants, this, by no means, qualifies as 'mission accomplished' with regard to getting these products to market and, more importantly, into the hands of anglers. Now, in my head at least, if Duran Duran's *Hungry Like the Wolf* provided the uplifting soundtrack to the development and testing of Impulse™, then being brought back down to earth through the long slog of needing to launch a business dedicated to selling these products could very well be epitomized by another 80's classic - Soul II Soul's *Back to Life (Back to Reality)*. It turns out that transitioning between a research scientist and an entrepreneur is, for me at least, no easy task. Initially, I made the somewhat naïve assumption that once people found out about my *miracle* product, they'd be beating a path to my door. As a consequence, I simply put together what is now the aminotec.com website and Carpgeek TV YouTube channel. These outlets feature a host of scientific reports, educational videos and other information detailing the evolution of what would, ultimately, become our Impulse™ product line. These efforts were certainly not totally in vain, as they attracted interest from a number of enthusiastic anglers, as well as leading to the establishment of distribution deals with carpangler.com (USA) and Impulsesupplies.com (UK). Unfortunately, despite excellent feedback from the relatively small numbers of anglers who'd tried our products, the problem was just that – relatively small numbers. It became clear at this stage, in the spring of 2023, that we needed to spread the word to a much wider audience so, in concert with Mark Smith, my now trusted business partner and BioSource Baits (UK) co-founder, we got to work expanding

our operation. Briefly, we have developed a full range of Impulse™ Liquid, Paste and Gel products, each available in original (unflavored), fish, sweet cream and fruit flavors. These products are each designed to fit popular angling applications. Impulse™ Liquid is primarily intended for use with highly porous baits, such as pellets and groundbait. It can either be sprayed directly onto these feeds or diluted and used as a wetting agent or bait soak. Our Paste is simply created through directly blending our flavored base mixes with Impulse™ liquid, with these pastes most commonly finding use as boilie wraps. Finally, our Gels are designed for use as glugs or dips with harder, less porous baits such as particles or boilies. The Gel is also conveniently supplied in a condiment style squeeze bottle that permits direct injection into PVA bags. All Impulse™ products are PVA friendly and have extended shelf lives.



The products detailed above, when used correctly, are each designed to surpass the necessary threshold concentration of Impulse™ needed to induce a feeding response around the angler's bait. While, since 'breaking the dosage barrier,' this is now easier than ever to achieve, anglers should be careful to choose the right tool for the job. For example, giving a boilie hook bait a quick squirt with Impulse™ Liquid is inadvisable, as this small volume would almost certainly not be sufficient to establish the crucial concentration threshold and, in practical terms, would also very likely be washed off as the bait descended to the bottom. A better choice, as mentioned above, would be to use either a paste wrap or a gel dip, as these products provide a greater loading of Impulse™ while also remaining attached to the bait through contact with water. Conveniently, each product label contains its own QR code which, as soon as viewed through your phone's camera, links directly to a dedicated step-by-step instructional video at www.biosourcebaits.com detailing its preferred uses and full preparation instructions. A full downloadable user guide may also be viewed at the biosourcebaits.com website, along with other product-related features, full details on our complete product line, as well as a shopping cart for their purchase.

Are You Up to the Challenge?

As stated in the 'Want to Buy Some Golden Eggs?' section, developing a product that is proven to double catch rates is one thing, convincing anglers to take the plunge and spend their hard-earned cash on a new 'wonder bait' is something else. Thus, in order for anglers to try Impulse™ without incurring a significant expense, we have developed a range of cost-effective sample packs. Briefly, our "One Shot" test tubes, available for a couple of quid or less from Biosourcebaits.com, as well as other online stores and select retail outlets, provide the angler with enough Impulse™ to prepare a kilo of either pellets or groundbait. **Now, most importantly, anglers are both urged and recommended to *truly* prove to themselves that Impulse™ really 'does what it says on the tin' by using their One Shot samples to take what we call *The Impulse™ Challenge***. Essentially, the Challenge (see www.biosourcebaits.com for full details and instructions) has carp anglers simply fish with Impulse™ dosed baits on one rod, with otherwise identical undosed offerings fished on the other. Similarly, for pole fishermen, pairs of anglers can use comparable tactics by, for example, fishing adjacent pegs. This comparative method is essential, as no bait, no matter how effective, can catch fish that simply aren't there. Thus, by comparing catch rates between their two rods or poles, anglers are essentially only testing the single variable of bait attractiveness, just as was described earlier for the Sparsholt studies. If performed correctly, anglers should expect to see average catch rate differentials of ~2:1 or better. Finally, anglers are highly encouraged to report their experiences with Impulse™ through the Catch Reports area at the BioSource Baits website. In addition to offering monthly prizes for the best reported captures, this resource also provides more advanced hints and tips for getting the most out of the Impulse™ product range, as well as a host of tester reports and customer testimonials backing up the validity of our claims.

Lead tester Chris Pemberton with a fine brace of fish taken during field trials of Impulse™ gels and pastes

Back to the Future

As you may well have noticed, BioSource Baits isn't your traditional bait company – we are uniquely focused on the development and manufacture of novel amino acid-based fish feeding stimulants that, in turn, improve the fish catching qualities of virtually *any* bait they are applied to. This last sentence encapsulates the company's vision statement: *increase every angler's catch rate, regardless of the type of bait or style of fishing employed*. To this end, we are, based on customer feedback, continually tweaking our existing products, as well as developing new ones, to satisfy customer needs and expectations. Indeed, if you experience an 'I wish they sold this (other thing)' moment while using an existing Impulse™ product, please be sure to let us know! This process has already initiated the development of a powdered retail version of Impulse™, which we hope to bring to market towards the end of 2023.

One common request from customers is for the development of a line of traditional baits and feeds, such as boilies, pellets and groundbait, which already have Impulse™ encapsulated within them. This would, of course, render these products exceptionally user-friendly, as they could be used directly from the bag without any further fuss. However, the fishing bait market is an extremely competitive space – the last time I checked there were in excess of 100 boilie manufacturers operating in the UK alone. Thus, rather than take on this immense level of competition, it makes better sense to provide these existing bait companies with our additives for use within their own products, so providing *them* with a much-needed competitive edge. To this end, we have developed a range of business-to-business products, essentially bulk versions of our impulse liquids and powders, which are available through a dedicated trade area at the BioSource Baits website. Importantly, we also offer a complementary consultation service for B2B clients wishing to either incorporate Impulse™ into their own products or to collaborate with us to develop something completely new. Good companies generally listen to their customers' needs so, if you'd like to see an Impulse™ infused version of your favorite bait on the shelves of your local tackle shop, then I'd certainly urge you to hit up the respective manufacturer(s) through their socials – the more demand there is, the more likely we are to get that company's phone call. The second collaborative option just mentioned offers some particularly exciting possibilities. In more detail, based on the fact that the AAs contained within any Impulse™ product must quickly exceed and maintain a post-critical threshold concentration in and around the baited area to be most effective, it would further make sense that any such optimized products would also be efficient at facilitating this process. In my mind, I see the way forward in this area through the development of what I like to refer to as 'depth charge' embodiments – feed products that essentially sink to the bottom prior to 'exploding' Impulse™ into the baited area. While we have gone some way to addressing this with the creation of our line of pastes, other, perhaps more effective options, include soluble boilies and fast breakdown pellets created using an Impulse™ infused base mix, as well as the creation of hydrolysate liquids rendered stimulatory, as discussed previously, through the addition of Hydrostim™ 'site-blockers'. While these latter two, more challenging, embodiments are likely best left to the bait companies, **anglers who roll their own baits are highly encouraged to create soluble 'depth**

charge' variants by using neat impulse™, instead of water, to make soluble versions of their homemades.

Thank you for reading this report. It's been quite a journey for that wide-eyed teen from the 1980's who became spellbound by and, let's be honest, probably a little too obsessed by *Carp Fever*. If I were somehow able to pull a Biff Tannen and smuggle an amino acid almanac back to my teenage self, then, before returning back to the future, I'd tell him, aside from don't take physics in college (sorry, still smarting from that), is that it's all worthwhile and you'll get there in the end - just try not to take half a lifetime trying to figure it out this time! To summarize it all, and without wishing to sound immodest, through cracking the amino acid code we have achieved what was once thought to be impossible – the creation of an effective, dosage blind amino acid-based fish feeding stimulant that better than doubles catch rates. We are convinced that products such as Impulse™ truly represent a 'Brave New World' and will ultimately revolutionize fishing as we know it and, as a consequence, will earn a place in every angler's bait bag.

Dr. Patrick Mills, June 2023

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